THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

ORDINARY MEETING

An Ordinary Meeting of the Society was held at 2.30 p.m. on Wednesday, November 2nd, 1960, at the Wellcome Building, Euston Road, London, N.W.1. The Chair was taken by the President, Mr. R. C. Chirnside, F.R.I.C.

The subject of the meeting was "Analysis of Semi-conductors" and the following papers were presented and discussed: Introductory Remarks by C. A. Parker, B.Sc., Ph.D., F.R.I.C.; "The Determination of Some Trace Impurities in Gallium Arsenide by Square-wave Polarography," by V. J. Jennings, B.Sc., Ph.D. (presented on his behalf by F. J. Bass, B.Sc., Ph.D.) "The Determination of Phosphorus in Silicon by a Fluorimetric Method," by R. E. Minns, A.R.I.C.; "Experiments on the Detection and Determination of Impurities in Silicon by Means of Gas Chromatography," by J. E. Still, B.Sc., F.R.I.C., and R. C. Chirnside, F.R.I.C.; "The Determination of Boron in Silicon by Isotope Dilution," by D. C. Newton, B.Sc., J. Sanders, A.I.M., and A. C. Tyrrell; "The Spectrographic Analysis of Trace Impurities in Indium, following Chemical Concentration," by J. F. Duke, B.A., H. R. Whitehead, B.Sc., and H. R. Sullivan; "Determination of Impurities in Semi-conductors by Spark-source Mass Spectrometry," by R. Brown and J. D. Waldron, B.Sc., Ph.D.; "Radioactivation Analysis," by J. A. James, M.A., B.Sc., A.R.I.C.

NEW MEMBERS

Ordinary Members

Muriel Joyce Ballard, B.Sc. (Lond.); Albert George Brown, B.Sc. (Lond.); Robert Stuart Bruce; Peter John Burnill, B.Sc. (Leeds); Fred W. Czech; Peter Owen Dennis, B.Sc. (Lond.) F.R.I.C.; Richard Hugh Doggett, A.R.I.C.; Peter Wolstenholme Elliott; Jeffrey Ellison, B.Sc., Ph.D. (Leeds); Henry Guy Harvey, M.Sc., Ph.D. (Lond.), F.R.I.C.; Alan Hewlett, B.Sc. (Lond.); Douglas Holness, B.A. (Cantab.); Michel Marcel Joerin, A.R.I.C.; John Samuel Jones, A.R.I.C.; David Thomas Lewis, B.Sc., Ph.D., D.Sc. (Wales), F.R.I.C.; Francisco Bermejo Martinez, D.Sc. (Madrid); Robert Hugh McKenna; Gladys Yvonne Pugh; Brian Esmond Russell, B.Sc., A.F.Inst.Pet.; Eric Shephard, B.Sc.(Lond.); Herbert Bowen Sotham; S. G. Tandon, M.Sc. (Lucknow); Giovanni Venturello; John Walker, L.I.M.; William John Wyse, B.Sc. (Lond.), A.R.I.C.

Junior Members

Brian John Bloice; David John Barrie Galliford; David Hewitson, B.Sc. (Liv.); Charles Peter Whyatt Lewins; Keith Gardiner Powell, B.Sc. (Manc.); Usha Priyadarshini, M.Sc. (Jabalpur); June Florence Scott; Henry Dixon Stuart.

DEATHS

We record with regret the deaths of

Alfred Bacon

William Marsden.

NORTH OF ENGLAND SECTION AND PHYSICAL METHODS GROUP

A JOINT Meeting of the North of England Section and the Physical Methods Group was held at 7.45 p.m. on Thursday, September 29th, 1960, in The Blossoms Hotel, Chester. The Chair was taken by the Chairman of the North of England Section, Dr. J. R. Edisbury.

The following papers were presented and discussed: "Applications of X-ray Spectrometry in the Oil Industry," by R. W. Toft, A.R.I.C., D.I.C.; "The Identification of Substances of Low Volatility by Pyrolysis - Gas Liquid Chromatography," by G. C. Hewitt, B.Sc., Ph.D., and B. T. Whitham, B.Sc., A.R.I.C.

The meeting was preceded at 2 p.m. by a visit to the Thornton Research Centre, "Shell" Research Ltd.

SCOTTISH SECTION

A JOINT Meeting of the Scottish Section with the Caithness Technical Society and the Polarographic Society was held at 9.45 a.m. on Friday, September 30th, 1960, in the Lecture Hall, Dounreay Experimental Reactor Establishment, Thurso, Caithness.

The Chairman of the Scottish Section, Mr. A. N. Harrow, A.H.-W.C., F.R.I.C., opened the meeting and took the Chair for the first session, when the following paper was presented and discussed: "Tesla-luminescence in Inorganic and Organic Systems," by R. J. Magee, M.Sc., Ph.D., A.R.I.C. At the second session, Mr. A. F. Williams, B.Sc., F.R.I.C., took the Chair, and the paper presented and discussed was: "Applications of Polarography in Industrial Analysis," by G. F. Reynolds, M.Sc., F.R.I.C. For the third session, Mr. Brian S. Dunn, M.Sc., A.R.I.C., representing the Caithness Technical Society, took the Chair and the paper presented and discussed was: "The Assay of Carbon-14 and Other Low-energy β-emitters," by J. C. Bevington, M.A., Ph.D., D.Sc.

At the conclusion of the meeting the visitors were taken on a tour of the Experimental Reactor Establishment.

MIDLANDS SECTION — ELWELL AWARD, 1960

The Elwell Award for 1960 was presented to D. J. Brindley at a meeting of the Section held at 6.30 p.m. on Tuesday, September 13th, 1960, at Regent House, St. Philip's Place, Birmingham, 3. The presentation was made by the Chairman of the Section, Dr. S. H. Jenkins, F.R.I.C., F.Inst.S.P. The runner-up was P. M. Owens.

The following papers, which had been submitted for the award, were presented: "The Polarographic Determination of Niobium in Highly Alloyed Steels," by D. J. Brindley; "The Identification of Vapour-phase and Liquid-phase Gums found in Gas Plant and Appliances," by P. M. Owens; "Moisture Determination in Carbon Dioxide Gas," by J. A. Roff.

MIDLANDS SECTION

AN Ordinary Meeting of the Section was held at 7 p.m. on Wednesday, October 12th, 1960, at the Lanchester College of Technology, Priory Street, Coventry. The Chair was taken by the Chairman of the Section, Dr. S. H. Jenkins, F.R.I.C., F.Inst.S.P.

The following paper was presented and discussed: "The Analytical Chemistry of Chromium and Vanadium," by G. M. Holmes, F.R.I.C.

BIOLOGICAL METHODS GROUP

A JOINT Meeting of the Biological Methods Group and the Pesticides Group of the Society of Chemical Industry was held at 7 p.m. on Wednesday, October 12th, 1960, in the Meeting Room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the Chairman of the Biological Methods Group, Dr. J. I. M. Jones, F.R.I.C.

The subject of the meeting was "The Biological Assay of Insecticidal Residues," which was introduced by J. H. Hamence, M.Sc., Ph.D., F.R.I.C., and the following papers were presented and discussed: "Biological Techniques for the Detection and Estimation of Insecticidal Residues," by P. H. Needham, B.Sc.; "Industrial Approach to the Biological Assay of Insecticidal Residues," by J. G. Reynolds, F.R.I.C., and R. Goulden, F.R.I.C.

The Application of Atomic Absorption to Chemical Analysis

A Review*

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SUMMARY OF CONTENTS

EARLY in the nineteenth century Fraunhofer observed many dark lines in the spectrum of the sun. Bunsen and Kirchhoff later showed that the positions of these lines corresponded with those in the emission spectra of certain chemical elements, thus making possible qualitative chemical analysis of stellar atmospheres by observation of atomic-absorption spectra superimposed on continua emitted from the central regions of the stars.

Approximate quantitative analysis of stellar atmospheres has since been achieved by empirically determining the oscillator strength of the transition giving rise to a line of an element; a closely controlled furnace containing atomic vapour of the element is used, through which is passed a suitable continuum such as that emitted by an incandescent tungsten lamp. The total absorption of the same line in a stellar spectrum is then measured, and the concentration of the element is estimated from theoretical curves relating total absorption to the number of absorbing atoms. Rosseland[1] gives a detailed account of the considerations involved in determining such "curves of growth," and Estabrook[2] describes the apparatus and technique used in measuring oscillator strengths of lines.

The phenomenon of atomic absorption has found wide application since the beginning of the century in research into physical aspects of atomic spectra. However, apart from its restricted use in such analyses as the determination of mercury vapour in the atmosphere, it was unknown among analysts as a means of chemically analysing terrestrial samples until Walsh,[3] in 1953, recognised its potential advantages over emission and devised apparatus that was sufficiently simple, versatile and inexpensive to be applicable to the routine analysis of solutions for a wide range of elements.

In 1955, Walsh[4] and Alkemade and Milatz[5] published papers describing the application of atomic-absorption spectra to chemical analysis. Walsh's paper surveys the theoretical factors involved in atomic-absorption analysis and gives the first published account of the advantages to be gained in the use of atomic absorption rather than emission in chemical analysis, these advantages being that atomic absorption is independent of the excitation potential of the transition involved and that it is less subject to temperature variation and interference from extraneous radiation or energy exchange between atoms.

Atomic-absorption analytical apparatus consists, essentially, of a suitable source of light emitting the line spectrum of an element, a device for vaporising the sample, a means of line isolation (monochromator or filter) and photo-electric detecting and measuring equipment.

* Reprints of this paper will be available shortly. For details, please see p. 856.

If the detector is placed to receive only the resonance line of the element from the light source, measurement can be made of the absorption of resonance-line radiation on its passage through the vaporised sample. The magnitude of this absorption gives a measure of the concentration of free ground-state atoms of the element in the vapour and, when referred to a calibration curve, provides a means of determining the concentration of the element in the sample.

Theoretical

The theoretical considerations of direct importance in the design and operation of atomic-absorption analytical equipment are given by Walsh.[4] Added general information is given by Mitchell and Zemansky[6] and in works connected with astonomical studies, such as Rosseland's.[1]

In low-temperature media of 2000° to 3000° K, such as in ordinary flames or furnaces, the population, N_o, of ground-state atomic vapour of an element is 10^4 to 10^{16} times the population, N_j, in the first excited state, depending on the temperature, T, the excitation energy, E_j, and the statistical weights of the states, P_j and P_o; thus—

$$N_j = N_o \frac{P_j}{P_o} e^{-E_j/kT}$$

The relatively large population of free atoms in the ground state compared with those at higher-energy levels predetermines the use of resonance lines, which arise from transitions ending in the ground state, in atomic-absorption analysis. For most metallic elements, these lines occur at wavelengths between 2000 and 9000 A.

Multiplets, resulting from closely spaced atomic-energy levels, exist in atomic-line spectra. If, as for sodium, there is only a single ground-state level but a multiplet upper level, all free ground-state atoms are capable of absorbing light of any component of the multiplet. However, if, as for the transition elements, a multiplet ground state exists, the number of atoms, N_v, available for a given transition is diminished according to the expression—

$$N_v = N \frac{P_i e^{-E_i/kT}}{\Sigma P_j e^{-E_j/kT}}$$

where N is the total number of atoms available, P_i and P_j are the statistical weights for the states involved and E_i and E_j are the excitation energies of the transitions involved.

The sensitivity of an atomic-absorption analytical method in which a line source is used is dependent not only on the concentration of atoms available for absorption, but also on the relative spectral widths of the line emitted from the source and the absorption line of the mass of atomic vapour. For maximum sensitivity the spectral half-width of the emission line should be very much less than that of the absorption line, so that peak absorption is measured.

The width of an absorption line is determined by the natural width due to the finite lifetime of an atom in the excited state, Doppler broadening due to motions of the atoms, Lorentz broadening due to collision with foreign gases, Holtsmark broadening due to collision with atoms of the same kind and Stark broadening due to electric fields, either internal due to ions and electrons or external. Of these, Doppler, Lorentz and Holtsmark broadening significantly contribute to line width at low vapour pressure of an element in ordinary flames or furnaces, Doppler broadening probably predominating. Doppler broadening produces a line width of the order of 0·01 A at 2000° K and is given by the expression—

$$D_\lambda = 1{\cdot}67 \frac{\lambda}{c} \sqrt{\frac{2RT}{M}}$$

where λ is the wavelength, R the universal gas constant and M the atomic weight.

The same factors, as well as broadening due to self-absorption, control the width of an emission line. For maximum sensitivity in analysis, the aim is, therefore, to operate the emission source at as low a temperature and pressure as possible and to minimise the path length of light through the non-emitting regions of the source where self-absorption can occur.

If the half-width of the emission line is negligible compared with that of the absorption line, and if the shape of the latter is determined entirely by Doppler broadening, the relationship between absorption coefficient, $K_{max.}$, and concentration is given by—

$$K_{max.} = \frac{2\lambda^2}{D_\lambda}\sqrt{\frac{\ln 2}{\pi}} \cdot \frac{\pi e^2}{mc^2} \cdot Nf$$

where D_λ is the Doppler width, N the number of absorbing atoms per unit volume and f the oscillator strength of the transition involved. This implies linear relationship between absorption and concentration, which is only approached in practice at low concentration. At high concentration, the width of the line is principally determined by factors other than Doppler broadening.

Instrumental

Light sources—

For the more volatile elements, such as the alkali metals, mercury and thallium, the most convenient source is the spectral vapour lamp, which consists of a closed glass or silica tube, into which are sealed oxide-coated electrodes, containing one of the rare gases and some of the appropriate metal. They are generally operated on alternating current, a transformer or choke being used. Elenbaas and Riemens[7] describe the construction, characteristics and operation of such lamps, which are freely available commercially. According to Russell, Shelton and Walsh,[8] improvement in sensitivity is effected by operating these lamps at currents considerably lower than those recommended by the manufacturers.

The alternative line source for volatile elements or elements having volatile halides is the high-frequency electrode-less discharge lamp. These, however, are stated[8] to have less-stable light output and to require a much more expensive power-pack than spectral vapour lamps.

Although flames are well known sources of line spectra, their use as light sources in atomic-absorption analysis would be less satisfactory than use of other line sources because of line broadening due to high operating temperature and pressure, their inability to excite lines of high excitation potential and their probable unstable light output over long periods.

For line sources of the less-volatile elements, hollow-cathode discharge tubes have been found the most satisfactory. These consist, essentially, of an anode and hollow cylindrical cathode (either composed of or lined with the appropriate metal) mounted in a sealed glass tube containing one of the rare gases. In operation, bombardment by rare-gas ions causes free atoms to be sputtered off the cathode. These are excited by collision with rare-gas atoms and emit a strong line spectrum from the confined space inside the cathode where their concentration is high.

Hollow-cathode discharge tubes developed for high-resolution spectroscopy have been of the vacuum-circulating type, in which impurities owing to outgassing are removed by appropriate traps, or have contained a getter consisting of a metal that will remove gaseous impurities by reacting with them. Activated uranium[9] and zirconium[8] are examples of getters suggested for hollow-cathode discharge tubes. However, Jones and Walsh[10] have recently found that it is clean-up, probably owing to sputtering causing absorption of the rare gas in the electrodes, that impairs the life of these tubes rather than impurities outgassing from the electrodes. They have found that, with no getter or circulating system, a tube life of 3 ampere-hours at a volume of 75 ml is increased to 50 ampere-hours at 250 ml, and they recommend the latter volume. Detailed information on the design and construction of hollow-cathode discharge tubes for atomic-absorption analysis is given by Jones and Walsh[10] and Lockyer.[11] They are manufactured commercially in England by Hilger and Watts Ltd. and in Australia by Ransley Glass Instruments Ltd., Melbourne.

From the point of view of freedom from carrier-gas lines in the region of the spectrum where most resonance lines exist and of obtaining maximum light output at a given tube current, neon[10] is the best rare gas with which to fill hollow-cathode discharge tubes. However, a lower clean-up rate found[10,12] for argon makes it preferable to neon as a carrier gas when a monochromator is used for resonance-line isolation, particularly since there is little difference, at least for calcium tubes,[12] in sensitivity in absorption if one or the other rare gas is used. The use of neon would probably give greater sensitivity[10] than argon if filters are used for line isolation, because their wider band pass would allow unabsorbed light of the

argon spectrum to reach the detector. According to Lockyer,[11] the low atomic weight, resulting in low sputtering power, and the tendency to excite spark rather than arc lines makes helium less satisfactory than heavier rare gases in hollow-cathode tubes.

When hollow-cathode tubes break down due to clean-up of the rare gas, they can be restored to operation by repeated evacuation, filling with spectrally pure rare gas and running at 100 to 150 mA on a high-vacuum line until clean, then sealing off. This procedure takes about 6 hours, but several tubes can be serviced simultaneously on the vacuum line. Practice[10] to date has been to use a silicone 702, two-stage diffusion pump, mechanical backing pump, differential manometer and liquid-air trap in the line. Lockyer[11] claims that a rotary vacuum pump alone would be all that is required, but gives no details of procedure. A tube is judged ready to seal off from the vacuum line when the rare-gas pressure (generally about a 2-cm head of silicone oil) and purity are such as to give steady and uniform emission of light from the hollow cathode, which, when viewed through a hand spectroscope, shows a sharp line spectrum free from molecular bands or background. An interesting appraisal of the effect of tube background on analytical sensitivity is given by Allan.[13] He found that high continuous background emitted from a manganese tube caused 35 per cent. of the light passing 0·1-mm wide slits of a monochromator set at 2794·8 A to be not absorbable by the atomic vapour of manganese. Photographic detection and microphotometric measurement with this tube or photo-electric measurement with a background-free manganese tube gave equal and much higher analytical sensitivity.

Line spectra of several elements can be obtained from a single tube either by using an alloyed cathode or several pure-metal hollow electrodes[10] sealed into the tube, the metal appropriate to the spectrum required being selected as cathode.

In the application of atomic-absorption methods to chemical analysis both smoothed direct current[4,8,13,14,15] and half- or full-wave rectified alternating current[12,16,17] have been used to supply hollow-cathode tubes. The use of rectified alternating current or smoothed direct current and a mechanical chopper[4,5,8] in the light path between the hollow-cathode tube and vaporised sample results in an alternating signal from the detector. If this signal is measured by using an a.c. amplifier tuned to the frequency of the signal, the system is specific to measurement of light emitted from the hollow-cathode tube. Box and Walsh[18] describe in detail the system based on an a.c. amplifier and hollow-cathode beam modulated by the use of rectified alternating current.

If a double-beam system, such as that described by Russell, Shelton and Walsh,[8] is used, stabilisation of the voltage applied to the spectral lamp is unnecessary. If, however, one of the simpler single-beam systems[13,15,18] is used, stabilisation is necessary to ensure constant light output from the spectral lamp so that blank, sample and standard are measured under identical conditions. Satisfactory stability has been obtained by supplying the power-pack of the spectral lamp from conventional commercially available electronic voltage-stabilisers delivering either 230 volts ($\pm$0·25 per cent. for $\pm$10 per cent. mains variation), 50 cycles per second a.c.[16,17,18] or 1150-volts d.c.[14]

The no-load output voltage of hollow-cathode power-packs should be about 800 volts[14,18] and the output current variable between 5 and 100 mA. Tubes are operated at the lowest possible current consistent with stable cathode emission and adequate signal-to-noise ratio from the resonance-line detector.

VAPORISATION OF SAMPLE—

With the exception of mercury, which produces a considerable concentration of monatomic vapour in the atmosphere at room temperature, it is necessary to use some artificial means of converting the sample into ground-state atomic vapour. For the purpose of atomic-absorption analysis, this has been achieved both by heating and by electrical means.

Furnaces, such as those used in astronomical investigations,[2] have not been employed in atomic-absorption analytical apparatus, probably because they are not conducive to quick interchange of samples and would be slow in reaching equilibrium.

Atomic-absorption methods have been applied almost exclusively to the analysis of solutions and for this purpose flames, fed with a fine spray of the sample solution, similar to those employed in flame photometry are used. Allan[14] and David[16] have used Lundegårdh sprayer - burner assemblies operating on an air - acetylene mixture. The E.E.L. (Evans Electroselenium Ltd.) system, modified to operate on an air - acetylene mixture, has been used by Willis[17] and David.[19] Russell *et al.*[8] and Lockyer and Hames[20] used air - coal gas

mixtures, and Malmstadt and Chambers[21] used an air - propane mixture. In a study by Willis[17] of sensitivity in the determination of calcium in blood serum, an air - acetylene flame was found superior to oxy-acetylene, oxy-hydrogen or air - coal gas flames. Since some calcium compounds are difficult to dissociate thermally,[12,17] an air - acetylene mixture would probably be found universally superior to mixtures of other gases if both convenience and sensitivity are considered.

In the determination of calcium in solution with an air - acetylene flame David[12] found that the sensitivity (*i.e.*, the concentration of ground-state atomic vapour of calcium) increased at the base of the flame if the only anion present was chloride. If, however, certain other anions were present, notably phosphate, maximum sensitivity was observed at a higher point of intersection of hollow-cathode beam and flame. This effect, which is more marked with calcium than with other elements, is obviously the result of the formation of temperature-stable compounds in the flame. Willis[17] has found the effect much more pronounced if an air - coal gas flame is used, it then being necessary to pass the beam from the calcium hollow cathode very close to the base of the flame to obtain any appreciable absorption. Since this effect would be different for different burners and different gas mixtures, it is advisable to test a burner not previously used in atomic-absorption analysis for variation in sensitivity with height of intersection of hollow-cathode beam and flame.

A further method of gaining sensitivity by adjustment of the burner is to increase the length of path of the hollow-cathode beam through the flame[8] either by use of a long burner of the fish-tail type or by multiple reflection of the hollow-cathode beam with front-faced aluminium mirrors. Russell *et al.*[8] and Allan[13] have shown that both methods give gain in sensitivity approximately proportional to the path length of the beam in the flame. Allan[14] has found that burning back occurred if an air - acetylene burner longer than 12 cm was used.

Some elements, such as aluminium and silicon, though they have resonance lines of suitable wavelength, cannot be determined by atomic-absorption methods with conventional flames for vaporising the sample because the refractory nature of their oxides prevents the production of sufficient free atoms for measurement. Vaporisation of the sample in a non-oxidising high-temperature environment, such as that produced by the plasma jet described by Margoshes and Scribner,[22] may make possible the determination of these elements in solution by atomic absorption.

The only atomic-absorption method so far investigated for samples other than in solution is that proposed by Gatehouse and Walsh[23] in which a metallic sample is made to produce monatomic vapour by sputtering. A cylinder, open at both ends, machined from the alloy to be analysed is made the cathode of a demountable hollow-cathode discharge tube through which light can be passed by provision of suitable windows. Pumping, filling with a rare gas and passage of a discharge produces monatomic vapour of the alloy, and the concentration of a given element in the alloy can be determined by measuring the absorption of resonance-line radiation of that element when it is passed through the atomic vapour. Stated advantages of the method are its use of samples in the metallic state, its avoidance of interference due to chemical combination, its possible application to elements whose strongest resonance lines are in the vacuum ultra-violet region and the ease with which sensitivity can be varied by varying the discharge current. Gatehouse and Walsh have successfully applied the method to the determination of 0·005 to 0·05 per cent. of silver in copper.

Line isolation—

The use of a line spectrum of the element being determined rather than a continuum as light source makes possible the use of monochromators of low resolving power or even filters. Walsh[4] states that, if a continuum were used, a monochromator with resolving power of at least 500,000 would be necessary to isolate a spectral width of less than the half-width of the resonance line of the element being determined. If, however, a spectral lamp is used as light source, it is only necessary to isolate the resonance line from neighbouring lines of the light source or vaporised sample.

The resolution required can be lowered even further by the use of a modulated source and a.c. amplifier tuned to the frequency of modulation.[4,5,18] Since, with this system, light from the vaporised sample falling on the detector produces a d.c. signal that is rejected, it is only necessary to isolate the resonance line from neighbouring lines emitted from the source. The resolution then required depends only on the complexity of the spectrum emitted from the source. For the transition elements, a resolution of the order of 1 A would be necessary

to isolate the strongest resonance lines; for the alkalis and alkaline earths, filters similar to those used in flame photometry are satisfactory. Since practically all light emitted from a sodium spectral lamp is concentrated in the resonance doublet at 5890 - 5896 A, it is possible to determine sodium by atomic absorption without the use of either monochromator or filter[24,25] if a modulated lamp and a.c. amplifier are used.

The determinations in solution of zinc, iron, manganese, sodium, potassium, calcium and magnesium by atomic absorption have been performed with Hilger medium-quartz spectrographs, with exit slits and photomultipliers attached, by Allan[13,14] and David.[12,16,19] Willis[17] used a Beckman DU monochromator for calcium, magnesium, sodium and potassium in blood serum, and Lockyer and Hames[20] a Uvispek H700 for determining noble metals in solution. Malmstadt and Chambers[21] used combinations of filters for isolating the sodium and potassium resonance lines.

Russell and Walsh[26] have drawn attention to the possibility of using a hollow-cathode discharge tube as a resonance lamp,[6] thus dispensing with the necessity of using monochromators or filters for selecting absorbing lines. Chromium and copper spectra published by them show that, whereas light emitted axially from a hollow cathode is composed of lines ending in many atomic-energy levels, that emitted at right angles to the axis from atomic vapour close to the front of the cathode consists only of chromium and copper resonance lines and weak rare-gas lines. This method of isolating resonance lines has not been used in analysis, but it is pointed out that the lack of intensity at right angles to the axis would be, to some extent, compensated for by avoidance of the intensity restrictions imposed by monochromators.

DETECTION AND EVALUATION—

Photo-electric detectors used in atomic-absorption analysis need be no more sensitive than those used in emission analysis because, in the atomic-absorption method, concentration of an element is assessed by measuring the reduction in intensity of the resonance line emitted from a source of high intensity. In emission analysis, on the other hand, sensitivity at low concentration is limited by the detector failing to give adequate signal-to-noise ratio or failing to measure a spectral line intensity, which is small compared with background intensity.

The resonance line of lowest wavelength so far used in atomic-absorption analysis is that of zinc at 2138 A[8,16] and the highest that of caesium at 8521 A.[8] Between, and including, these wavelengths, R.C.A. 1P22 photomultiplier tubes for the visible and near-infra-red regions and R.C.A. 1P28 for the ultra-violet and visible regions have been used successfully. However, there is no reason why other photomultipliers should not be as satisfactory. Photocells[20] and cadmium sulphide photoconducting cells[21] have been found to operate satisfactorily at a variety of wavelengths between these limits. Extension to higher and lower wavelengths could be achieved by the use of photomultipliers or photocells responding in the appropriate spectral region, fluorescent coatings, such as sodium salicylate or cadmium sulphide, or other photoconducting cells. Information on sensitivities and ranges of response of a wide variety of photosensitive detectors is available in the manufacturers' literature.

The d.c. measuring systems that have been used in evaluating signals from photoelectric detectors are a galvanometer provided with a variable shunt and small opposing e.m.f described by Allan,[14] the single-beam null-point system of the Hilger Uvispek employed by Lockyer and Hames[20] and a single-beam null-point system devised by Malmstadt and Chambers,[21] which is unusual in that it is based on the matching of the concentration of the standard solution to that of the sample solution rather than the matching of e.m.f.'s with a bridge or of light intensities with a variable diaphragm. Although it is claimed to be more precise than other single-beam systems, it would be more difficult to operate because a standard must be volumetrically adjusted to null point with each sample solution analysed.

Walsh[4] and Alkemade and Milatz[5] have described systems based on a.c. measurement. Box and Walsh[18] later developed a simple single-beam instrument based on a.c. measurement, which has been applied to chemical analysis of various materials by Russell *et al.*,[8] David,[12,16,19] Willis,[17] Brownell,[25] and Gatehouse and Walsh.[23]

The principal factor now limiting sensitivity of the atomic-absorption method is fluctuation of the light source. The conventional method of overcoming such fluctuation is that of double-beam operation. Russell *et al.*[8] used a double-beam system not originally designed for atomic-absorption spectroscopy and which would be too complex and expensive for general use in routine analysis. Alkemade and Milatz[5] described a double-beam instrument employing

a light filter for line isolation in which the beams were mechanically modulated in opposite phase. Menzies[27] and his co-workers state that the use of the ratio of readings from two channels of a direct-reading medium-quartz spectrograph, one of which is placed to measure the absorbing copper resonance line at 3247 A and the other the non-absorbing copper line at 2824 A, shows promise in eliminating the effect of fluctuation in light output from a copper hollow-cathode discharge tube. Investigations have been carried out by Box and Walsh[18] into simple methods of monitoring the light source, which may lead to great gains in sensitivity.

Any of the well known commercially available spectrophotometers can be adapted to atomic-absorption analysis based on the d.c. measuring system. An atomic-absorption attachment for a spectrophotometer, which will allow atomic-absorption measurement to be carried out by the d.c. system, is available commercially.[15] Power-packs for hollow-cathode tubes and combined photomultiplier high-voltage supply - a.c. amplifier units for atomic-absorption measurement by the a.c. system of Box and Walsh[18] are also available (Techtron Appliances Ltd., Melbourne, Australia).

Comparison of Atomic Absorption with Other Analytical Methods

Sensitivity—

For an air - acetylene flame of length 2 to 3 cm the lower limits of detection of elements having low resonance-line excitation potential are approximately equal in single-beam atomic absorption and emission methods. For example, the limits both in absorption[19] and emission,[28] under the conditions stated, lie between 0·01 and 0·1 p.p.m. in solution for both sodium and potassium. Since these limits are adequate, according to Allan,[14] for most analytical purposes little would be gained, with respect to sensitivity, by using atomic absorption rather than emission. However, occasionally, for example, when only small amounts of sample are available, the gain in sensitivity possible in the atomic-absorption method by increasing the length of flame pass of the beam from the spectral lamp would give it the advantage over emission methods.

For elements having highly reversed resonance lines or resonance lines of high excitation potential, the atomic-absorption method has decided advantages over emission methods. Examples of elements in these categories are zinc, magnesium, iron and manganese. David,[16] who used a 2-cm air - acetylene flame, has found the lower limit of determining zinc in plant-digest solutions to be 0·5 p.p.m., whereas, owing to insensitivity, flame-emission methods for zinc in plants are unknown. The strong self-absorption and high excitation potential of the magnesium resonance line at 2852 A give it a poor response to concentration and a limit of determination of about 5 p.p.m. in solution by flame emission,[28] whereas Allan,[14] David[19] and Willis[17] have shown the atomic-absorption limit to be 0·1 p.p.m. or less in solution, depending on the length of flame pass of the hollow-cathode beam. Allan,[13] who used the iron line at 2483 A and the manganese line at 2795 A, showed that an optical density of 0·1 could be produced in a 12-cm air - acetylene flame by 2·0 and 1·3 p.p.m. of iron and manganese, respectively, in solution. This would give a limit of determination by atomic absorption of about 0·1 and 0·05 p.p.m. in solution for iron and manganese, respectively, which is about a 50- and 5-fold improvement, respectively, on the Lundegårdh flame-emission method.[29] The lines used in the Lundegårdh method quoted were Fe 3560 A and Mn 4031 A, which are lines of low excitation potential. As has been pointed out by Allan,[13] the strongest emission lines are not necessarily the strongest in absorption for elements having complex spectra.

A slight procedural disadvantage of the atomic-absorption method, when compared with flame emission, is the lack of a quick and simple method of varying sensitivity to deal with solutions of widely varying element concentrations. The reason for this is that, whereas the sensitivity of flame-emission instruments can be varied merely by varying the gain of the measuring unit, that of an atomic-absorption instrument is determined almost entirely by flame characteristics, notably length of light path through the flame. When the absorption reaches 80 per cent. or more, the slope of an atomic-absorption calibration curve is such that small errors in the absorption readings produce large errors in the determination. The only methods of lowering the absorption to the sensitive part of a calibration curve are dilution of the sample or reduction in length of the light path through the flame by changing the burner or varying the number of passes of the light beam through the flame if a multi-pass system[8] is used. Since accurate analyses can be carried out over a concentration range of about

40-fold[16] with a given burner, this difficulty would not arise in routine analysis in which the approximate composition of samples is known. If, however, the approximate composition is not known, it is necessary to carry out a rough preliminary run to determine the appropriate dilution factor for a sample. A similar difficulty, which apparently does not cause serious inconvenience, is encountered in colorimetric analysis.

For elements that can be determined colorimetrically, the atomic-absorption method is often less sensitive than a colorimetric method. The difference is, according to Allan,[13] sufficiently small for iron and manganese to give the atomic-absorption method advantage on grounds of rapidity and simplicity. For copper, however, the absorption of the resonance line at 3247 A in a 2-cm air - acetylene flame was not found great enough to allow direct analysis of plant-digest solutions with a single-beam atomic-absorption instrument,[16] whereas colorimetric methods for copper[30,31] in these solutions are adequately sensitive. An examination of the determination of copper in plant-digest solutions by atomic-absorption methods in the light of suggested means of gaining sensitivity may meet with success.

It has been shown by David,[16] that, even with an air - acetylene flame approximately 2 cm long, the atomic-absorption method for zinc is more sensitive than are polarographic methods and has the added advantage of being less complicated.

Willis[17] found that an accurate determination of magnesium could be carried out by atomic absorption on 0·1 to 0·2 ml of blood serum, whereas reliable gravimetric or volumetric methods required at least 1 ml.

In considering the sensitivity of atomic-absorption methods it is important to realise that practically all investigations to date have been carried out with single-beam instruments. Future instrumental advances, such as the introduction of simple double-beam systems and scale-expansion devices are likely to effect large gains in sensitivity.

TABLE I

SENSITIVITY AND PRECISION OF DETERMINATIONS OF CERTAIN ELEMENTS IN SOLUTION BY ATOMIC ABSORPTION

Element	Resonance line, A	Range of measurements, p.p.m.	Coefficient of variation at concentration (p.p.m.) given in brackets, %	Flame gases* and length, cm	Reference†
Na	5890/6	1 to 10	—	A-C, 3	25
		0·1 to 50	5·0 (0·5); 0·9 (5)	A-A, 2·7; A-C, 2	8, 19
		0·03 to 100	5 (1); 0·6 (5); 0·44 (100)	A-P, 2·6	5, 21
K	7665/99	0·1 to 100	7·5 (1); 1·8 (10)	A-A, 2·7; A-C, 2	8, 19
		1 to 100	1·5 (2); 1·6 (5); 0·4 (100)	A-P, 2·6	21
Cu	3247	25 to 50	1·0 (25); 0·3 (50)	—	41
		1 to 100	—	A-C, 2	8
Rb	7800	2 to 100	—	A-C, 2	8
Ag	3281	2 to 100	—	A-C, 2	8
		0·1 to 10	7 (0·5); 0·5 (10)	A-C, 10	20
Cs	8521	10 to 100	—	A-C, 2	8
Au	2428	2 to 100	—	A-C, 2	8
		1 to 50	14 (1); 0·2 (50)	A-C, 10	20
Mg	2852	0·1 to 100	1 (0·3); 6·6 (0·3); 2 (3)	A-A, 10, 2·7, 7·5; A-C, 2	8, 14, 16, 17, 19
Ca	4227	0·2 to 50	1·0 (10); 4 (2·5); 2 (50)	A-A, 10, 2	12, 17, 19
Zn	2139	0·1 to 100	13 (1); 2 (10)	A-A, 2; A-C, 10, 2	8, 16, 41
Cd	2288	0·1 to 100	—	A-C, 2	8
Tl	2767	10 to 100	—	A-C, 2	8
Pb	2833	100 to 200	—	A-C, 10	41
Cr	4254	40 to 200	—	A-C, 2	8
Mn	4031	10 to 75	—	A-C, 10	41
	2795	0·5 to 25	8·8 (0·5); 4 (25)	A-A, 12	13
Fe	3720	5 to 200	—	A-C, 2	8
	2483	2·5 to 125	6·5 (2·5); 3·3 (125)	A-A, 12	13
Ni	3415	10 to 100	2 (50)	A-C, 2, 10	8, 41
Rh	3435	2 to 100	17 (2); 0·2 (100)	A-C, 10	20
Pd	2476	2 to 100	6 (2); 0·4 (100)	A-C, 10	20
Pt	2659	10 to 100	30 (10); 1·2 (100)	A-C, 10	20

* A-A = air - acetylene
A-C = air - coal gas
A-P = air - propane.
† See reference list, p. 790.

The ranges of determination for a number of elements by atomic absorption reported in the literature are given in Table I.

PRECISION—

The precision of the single-beam atomic-absorption method appears to be principally dependent on the stability of light output from the spectral lamp. This is, in turn, dependent on the stability of the mains supply and the inherent stability of the lamp. Efficient voltage-stabilisers are available commercially for control of fluctuation in mains voltage, but inherent fluctuation, being a property of the lamp itself, cannot be eliminated. Fortunately, the worst of these fluctuations is not great, being less than ± 2 per cent. for a zinc hollow-cathode tube or potassium spectral vapour lamp. Since a mean position can be assessed in setting full-scale deflection or taking a reading, this inherent fluctuation would probably not produce an uncertainty of more than 0·5 per cent. in practice. Some hollow-cathode tubes, notably calcium and magnesium, show neither perceptible short-term fluctuation nor drift over a period of operation of about an hour, if in good condition.

When a hollow-cathode tube is nearing the end of its life, downward drift in light output occurs. Correction can be made for this, if not too great, in setting full-scale deflection. However, since long life can now be expected of hollow-cathode tubes,[18] this difficulty will seldom arise.

The other sources of fluctuation in the atomic-absorption method, to which flame-emission methods are also subject, arise from the flame and the detector and measuring unit. According to Walsh,[4] fluctuations in absorption resulting from fluctuations in flame temperature should be much less than those in emission because the strength of an absorption line is principally dependent on Doppler width, which varies only as $T^{\frac{1}{2}}$, whereas the intensity of emission of a line from a flame is very sensitive to temperature. There is probably little difference between fluctuation in single-beam atomic-absorption and emission methods resulting from instability in the detector and measuring unit, and, in any case, these fluctuations would be negligible compared with those from other sources.

It is to be expected, then, that the precision of the single-beam atomic-absorption method would vary from one element to another depending upon the stability of the light source, whereas that of photo-electric flame-emission methods would be approximately constant for different elements, their precision being most dependent on flame stability. The best atomic-absorption measurements should, therefore, be more precise than photo-electric flame-emission measurements. These generalisations seem to be borne out by comparison of atomic-absorption coefficients of variation reported in Table I with published coefficients of variation of photo-electric flame-emission methods. Oertel and Stace[32] found the contribution of flame fluctuation to the over-all error in the Lundegårdh method to be 2 per cent. for potassium, 1·5 per cent. for sodium and 1·5 per cent. for calcium; later work by Stace[28] indicated coefficients of variation in a flame-photometric method to be 1 per cent. for sodium and 1·2 per cent. for potassium. Results of other workers by flame-photometric methods are in agreement with these.

Absorptiometric methods, generally, are more precise than single-beam atomic-absorption methods because greater stability exists both in the light sources used and in the media in which absorption measurements are made. A precision of 0·2 per cent. in determining transmittance is attainable according to Sandell.[33]

The precision of an analytical method can be assessed, to some extent, by observing the magnitude of departure of calibration points from a smooth curve. Atomic-absorption calibrations, based on single readings for each point,[16] show no departure from a smooth curve for magnesium and only slight departure for zinc.

ACCURACY—

In flame-emission analysis, three types of interference from extraneous elements in the sample are possible. These are—

(*a*) *Physical*—Excitation energy is transferred by collisions of the first or second kind[34] in the flame between atoms of different species, atoms and electrons or atoms and molecules, causing enhancement or depression of analysis-line emission. This type of energy transfer is a common phenomenon in discharge tubes,[6] but, although it almost certainly exists in flames, it has not been identified separately from other types of interference in flame-emission analysis.

(*b*) *Radiative*—Light from elements other than that for which the sample is being analysed passes the line- or band-isolating device (monochromator or filter), thus causing a positive analytical error. This type of interference is most common in filter flame photometers,[35] but is also known in flame-emission methods in which prisms or gratings are used for line isolation.[14] Common examples of radiative interferences in emission methods are those of potassium and calcium with the determination of sodium and sodium with the determinations of calcium and magnesium.

(*c*) *Chemical*—Emission of an element from a flame is depressed by the formation of compounds, which are not dissociated at flame temperatures, between the element and other ions in the sample solution. Examples of this type of interference are the depression of calcium and strontium emission[36] by aluminium and also of calcium emission[37,38] by phosphate, sulphate and silicate.

Whereas physical interference could cause inaccuracy in emission methods it has, according to Walsh,[4] no counterpart in atomic-absorption analysis because the number of absorbing atoms in a flame, on which atomic-absorption analysis is dependent, is so much larger than the number of excited atoms as to be effectively equal to the total number of atoms. Therefore, excitation-energy transfers, though they may have large effect on emission from a flame, have negligible effect on absorption in the flame.

The atomic-absorption method, whether based on a.c. or d.c. measurement, has distinct advantages over emission methods with respect to radiative interference. Allan,[14] who used a d.c. system, states that the electrical backing-off necessary to correct for background radiation from the flame (principally OH bands) near the magnesium resonance line at 2852 A is small in comparision with the signal from a magnesium hollow-cathode tube. He also draws attention to the fact that, whereas interference from the sodium line at 2853 A is detectable when the concentration of sodium reaches ten times that of magnesium in the Lundegårdh flame-emission method for magnesium, no interference by 17,000 p.p.m. of sodium can be observed in the determination by atomic absorption of 2 p.p.m. of magnesium.

If the atomic-absorption system based on a.c. measurement is used, absolute reliance can be placed on the elimination of all radiative interference of flame origin, whether it be due to the resonance line itself, flame background or emission from an extraneous element reaching the detector. The advantages of this system over atomic-absorption methods based on d.c. measurement and, particularly, over flame-emission methods are that samples of any composition can be analysed for a given element with impunity[19] and that large monochromator slit widths or wide-band-pass filters can be used without regard to extraneous light from the flame that may reach the detector.[5,24]

Unfortunately, atomic-absorption methods in which flames are used for vaporising the sample are subject to the same chemical interferences as are flame-emission methods, the reason being that the formation of a temperature-stable compound in the flame causes equal proportionate reduction in the populations of ground-state and excited atoms. For example, Strasheim and Nell's curves[39] showing interference of phosphate with calcium emission are similar in form to David's[12] showing its interference with calcium absorption; aluminium, which was shown to interfere with the flame-emission determination of alkaline-earth metals in solution by Mitchell and Robertson,[36] has been shown to interfere similarly with the atomic-absorption determination of magnesium and calcium by Allan[14] and David,[12] respectively.

Investigations to date suggest that serious chemical interference is confined, almost entirely, to the alkaline-earth elements and that calcium absorption is more subject to this interference than is magnesium absorption. In studying the application of atomic absorption to plant analysis, David[12,16] found that no serious interferences occurred in the determination of zinc, iron, copper or magnesium, but that the presence of phosphate, aluminium, silicate, sodium or potassium affected calcium absorption. In the analysis of soil extracts, David[19] found no interference with the determination of sodium or potassium, but interference from silicate, phosphate, aluminium and sulphate with calcium and magnesium absorption, magnesium being protected from interference if calcium were present. Willis,[17] in studying atomic-absorption analysis of blood serum, has found that no interference occurs in the determination of sodium, but that protein, sodium, potassium, magnesium and phosphate interfere with calcium absorption, protein with magnesium absorption and sodium with potassium absorption. Allan[13,14] has shown that aluminium can interfere with magnesium absorption,

but that no interference is evident from large excesses of potassium, calcium, sodium, magnesium or phosphate in the determination of manganese and iron by atomic absorption. In the determination of noble metals in solution by atomic absorption, Lockyer and Hames[20] found neither interference from large concentrations of iron and lead with gold, silver, platinum, rhodium or palladium absorption nor interferences from any one with the others. They did, however, find that if a water-cooled burner were not used, erroneous results were obtained through deposition of gold before it reached the flame. Menzies[27] reports that, whereas the presence of lead causes erratic emission results for copper and zinc in brass, due to segregation, the presence of 50 p.p.m. of lead has no effect on the determination of 0 to 100 p.p.m. of copper or zinc in solution by atomic-absorption methods.

The methods of overcoming chemical interferences in atomic absorption are similar to those used in emission, namely, either separation of interfering ions or suppression of the interference by addition to the solution of an excess of a substance that will prevent formation of compounds between the interfering ions and the element being determined. As is stated by David,[12] the latter method is preferable on grounds of simplicity of method and freedom from loss or contamination. Examples of substances added for this purpose, which have been used both in emission and atomic absorption, are strontium[17,19,36] and ethylenediaminetetra-acetic acid.[17,40] If the interference is positive or does not cause serious depression at high concentration of the interfering ion, it can be eliminated by adding an excess of the interfering ion to both sample and standard solutions. Sodium and potassium have been added to solutions for this purpose by David[12] and Willis.[17]

David[12] has found that 0·6 per cent. of magnesium and 2 per cent. v/v of sulphuric acid will together, but not separately, suppress phosphate, silicate and aluminium interference with the determination by atomic absorption of calcium in plant material.

The elimination of radiative interference by the use of atomic-absorption apparatus based on the a.c. measuring system allows the use of a much wider range of interference suppressors than is possible in emission flame photometry.

The wider range of elements that can be determined by atomic absorption than by emission gives atomic absorption greater overlap with colorimetric methods. There are, however, few quantitative data on which a comparison of accuracy of atomic-absorption and colorimetric methods can be based, and statements on the point must, therefore, be conjectural. As is stated by Allan,[13] atomic-absorption methods for iron and manganese are to be preferred to colorimetric on grounds of freedom from interference, rapidity and simplicity. Complicated extraction procedures are necessary in many colorimetric methods to remove interfering ions before the colour is developed. Such procedures, which are not essential to atomic-absorption analysis, must endanger the accuracy of colorimetric methods. For example, Sandell[33] quotes eight possible procedural sources of error in trace analysis by dithizone methods.

David[16] has compared atomic-absorption and polarographic methods for zinc in plant material and has found general agreement between the two methods. He claims, however, that the atomic-absorption method is preferable because it is simpler procedurally.

In metallurgical analysis, comparison has been made[41] between chemical and atomic-absorption results for copper and zinc in copper-based alloys. The results for copper agreed to within 0·5 per cent. and those for zinc to within 3 per cent.

If disagreement occurs between two methods in the analysis of a given set of samples, it is difficult to say which method gives the more accurate results, unless each is tested exhaustively for interferences. For this reason, David,[12,16,19] Willis[17] and Allan[13,14] have carried out recovery experiments by atomic-absorption methods in the analysis of a variety of samples for sodium, potassium, calcium, magnesium, iron and manganese, and found the results satisfactory. Willis[17] has analysed blood sera at various dilutions by atomic-absorption methods to test for interferences.

As well as reducing the possibility of loss and interference, the simplicity of procedure of the atomic-absorption method lessens the hazards to accuracy accruing from operator error and contamination.

Conclusions

The considerations that would most influence an analyst in choosing analytical equipment are range of elements to which it is applicable, sensitivity, precision, accuracy, cost and convenience in use. Since emission methods are established on these grounds in many laboratories, it is only necessary here to compare atomic-absorption with emission equipment.

It has been shown that atomic-absorption methods are as good as or better than emission methods, for elements to which they can both be applied, in sensitivity, precision and accuracy and, further, that atomic-absorption analysis can be applied to a far wider range of elements than can emission analysis.

The cost of atomic-absorption equipment is greater than that of flame-emission equipment, since, in addition to burner, monochromator and measuring unit, spectral lamps and hollow-cathode discharge tubes as well as the means of operating them must be provided. However, the extra cost should be more than compensated for by the greater range of analyses and greater reliability of results possible by atomic absorption.

Although the simultaneous determination by atomic absorption of several elements in a sample solution is possible by arranging spectral lamps along the focal curve of a spectrograph,[3] manipulative difficulties would probably be encountered in practice. These would arise from the fact that sensitivity in atomic-absorption analysis is determined almost entirely in the flame, whereas in emission analysis sensitivity can be set separately for each element by varying the gain of the measuring unit. The method is, however, conducive to routine analysis of solutions if each element is determined separately.

There is no sign, at the moment, of atomic-absorption equipment competing with the Quantometer in routine metallurgical analysis, because each sample needs considerable separate preparative treatment for atomic-absorption analysis. However, the very much lower cost of Gatehouse and Walsh's[23] equipment should make it attractive for direct non-routine analysis of metallurgical samples. The fact that the sensitivity of this equipment can be varied quickly and easily by variation of the current to the sputtering chamber should make it possible to determine several elements in a metallic sample in quick succession. Also, the elimination of chemical interference by vaporising a metallic sample in a sputtering chamber brings the absolute method of analysis mentioned by Walsh,[4] in which the concentration of an element is determined by direct calculation from the absorption reading rather than by reference to a calibration curve prepared from standards, into the realm of possibility.

The most likely future trends in the application of atomic-absorption spectroscopy to chemical analysis are the development of a simple and inexpensive instrument employing light filters for line isolation, the development of a simple double-beam system and the adaptation of the method to isotopic analysis. Isotopic analysis can be achieved[4] by the use of spectral lamps containing only the isotope it is desired to determine.

As has been shown, a filter instrument would probably be little more expensive than flame photometers at present available and would have the advantage of being free from radiative interference in analysis. Very large gains in sensitivity could be achieved by atomic-absorption equipment operated on the double-beam principle.

Addendum

Up to the proof-reading stage, some further publications on atomic-absorption methods have come to my notice.

A paper by Menzies[42] deals with the application of atomic absorption to chemical analysis generally. Allan[43] determines nickel and cobalt, the most sensitive absorption lines being the nickel line at 2320 A and the cobalt line at 2407 A, and David[44] determines molybdenum and strontium, use being made of the molybdenum line at 3132 A and the strontium line at 4607 A. A paper by Gidley and Jones[45] deals with the determination of zinc in metallurgical samples by atomic-absorption methods.

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An Investigation into the Use of Bioassay for Pesticide Residues in Foodstuffs*

Report to the Analytical Methods Committee by

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INTRODUCTION

THE Pesticides Residues in Foodstuffs Sub-Committee, with a view to the possible development of a rapid "sorting test" for detecting or determining toxic pesticide residues in food, required information on the extent to which bioassays are used for this purpose. During the investigation, laboratories in England, The Netherlands, Germany and Switzerland were visited. I acknowledge with gratitude these laboratories' permission to publish the information contained in this Report.

The laboratories were selected to include those of pesticide manufacturers, public analysts and official government and other institutes engaged in research or advisory work on pesticides and where tests in connection with approval schemes are made. One university department, at Göttingen in Germany, was visited.

Except for those of "Shell" Research Ltd. and J. R. Geigy A.G., the manufacturers' laboratories make little use of bioassay for residues. Some do not use bioassay at all, and others have techniques available that are used only when chemical assay methods for a particular pesticide are non-existent or give misleading results. These laboratories are, in the main, assaying samples known to contain a particular pesticide.

In England, I have not learnt of any public analytical laboratory in which bioassay is used for pesticides. On the Continent, however, I was able to visit two such laboratories: one in Amsterdam, the other in Zurich, and the workers in these regarded bioassay as an essential tool for their work. These laboratories are overworked, and biological tests are thought to be less time-consuming than chemical methods for sorting samples into those containing a pesticide residue and those not. They are also regarded by some as satisfactory methods of assaying the pesticide in the samples. It was emphasised, however, that bioassay alone is not satisfactory. It must be used in conjunction with chemical and chromatographic tests, these being used, for example, to identify the pesticides present, and the biological tests to assay them. From what has been learnt, it is evident that it is possible for bioassay tests to be made sensitive and accurate enough for this purpose.

The official government institutes visited are concerned with specific problems of contamination of food crops, arising from the doses at which they recommend the pesticides to be used. The Biologische Bundesanstalt laboratory, at Braunschweig, for example, was working at the time of my visit on the problem of aldrin and dieldrin residues in root crops, such as carrots and radishes. The East Malling laboratory is engaged on the specific problem of spray residues on fruit. These laboratories, like those of the manufacturers, are not attempting to assay residues of "unknown" pesticides.

The test organism most favoured by the laboratories visited for the bioassay of residues is *Drosophila melanogaster* (vinegar-fly). This insect is exposed either directly to a pulp of the sample to be tested or to a residual film left after the evaporation of the solvent from an extract solution. The direct or pulp test will not assay such small residues as will the test involving use of an extract unless the flies are exposed to the pulp for a very long time, sometimes for several days. When an extract is used, the extract solution can be concentrated by evaporation if the residue is small.

The larvae of *Aedes aegypti* (yellow fever mosquito) are used for bioassay in some laboratories. This is, I think, the most sensitive test organism in use for the bioassay of residues. It is used in an aqueous medium, with an extract of the sample to be tested in solution or suspension. The most serious drawback to the use of mosquito larvae is their extreme sensitivity to interfering substances in the extracts of most plant or animal materials.

* Presented at the joint meeting of the Biological Methods Group with the Pesticides Group of the Society of Chemical Industry on Wednesday, October 12th, 1960.

A. aegypti eggs may be stored for several weeks in a dry state; larvae can thus be obtained whenever they are required and not only when a rearing cycle makes them available.

Musca domestica (house-fly) and *Daphnia* spp. (water flea) are also used for residue bioassay. House-flies are used in the same way as *D. melanogaster*, and *Daphnia* spp. in the same way as mosquito larvae. There appears to be some difficulty over the rearing of *Daphnia*, although in the Institut für Pflanzenpathologie und Pflanzenschutz, at Göttingen, they are being reared with success, but not for bioassay.

Most of the test organisms used for bioassay appear to be relatively insensitive to one or more of the pesticides. For example, *D. melanogaster* is not easily affected by Sevin and is not affected by low concentrations of DDT; mosquito larvae are not affected by "residue" concentrations of DNOC. For this reason it is important to make use of more than one test species, particularly when investigating samples containing a residue of unknown identity.

An attempt has been made to find out the extent to which bioassay is used in the U.S.A. and in Canada for the determination of pesticide residues in foodstuffs. It would appear from replies to correspondence with certain laboratories in the U.S.A. that the use of bioassay is fairly extensive. University departments, industry, government laboratories (in which category are included the state laboratories) and private research laboratories are all engaged to some extent in this work.

The Universities of Arizona, Washington, Oregon and Cornell are all said to have workers dealing with bioassay of residues. The departments of Professor Hoskins in California and Professor Dewey at Cornell are probably doing the most work in this field.

The California Department of Agriculture has an operational residue bioassay laboratory, Colorado is in the process of setting up such a laboratory, and Nevada is said to be planning to do so in the near future.

The Food and Drug Administration have developed some techniques for residue bioassay that have been published, and their laboratories in Washington are obviously interested. Little information has been obtained, however, on the extent of this interest.

In industry, the laboratories of both the food-processing companies and the insecticide manufacturers use bioassay for residue determinations. One correspondent states that the baby-food canners are particularly interested in bioassay. Several large canning companies are said to apply bioassay techniques in connection with their regular quality-control programmes. Other such companies employ bioassay on a more limited scale. One large canning company was heard of that compares all its samples for bioassay with an arbitrary standard containing 0·1 p.p.m. of dieldrin. If the combined toxicity of the insecticides present in the sample is less than that of the standard, the material is assumed to be safe for their purpose.

Many of the insecticide manufacturers' laboratories use bioassay techniques for residue determinations in connection with the development of new compounds and new applications for existing ones. Among the companies using bioassay in this way are Shell, Du Pont, Monsanto and Dow.

The situation in Canada is not the same. On the government side, at least, there is little work going on specifically directed to the bioassay of pesticide residues in foodstuffs, although several establishments are engaged in the bioassay of small amounts of insecticides in connection with their research programmes. The techniques are used mainly for the study of comparative toxicities and not for residue determination.

The Food and Drug Directorate in Canada is interested in having bioassay procedures developed for the detection, identification and determination of pesticide residues in food and has suggested that the Department of Agriculture should collaborate in the work. Nothing has yet been done, although a study of organo-phosphate residues in potato tubers and brussels sprouts has been made under contract by the British Columbia Research Council.

VISITS TO LABORATORIES

"Shell" Research Ltd., Woodstock Farm, nr. Sittingbourne, Kent

(MR. A. RICHARDSON)

"Shell" Research Ltd. was the only laboratory visited in England in which bioassay was used as a routine measure for residues.

The bioassay technique employed involves use of the fruit-fly *D. melanogaster* Meig.,

but other suitable insects are reared and could be used if necessary. These are the house-fly *M. domestica* L. and the mosquitoes *A. aegypti* L. and *Anopheles gambiae* Giles.

The adult flies are exposed to a film of the extract left on the walls of a screw-topped 4-ounce jar after evaporation of the solvent from 2 ml of a solution. The jars, without their lids, are rotated in a horizontal position in the mouth of a fume cupboard, in such a way that evaporation of the extraction solvent leaves the residue distributed as evenly as possible over the inside surface. To avoid excessive evaporation of volatile insecticides that may be present in the extract, the jars are removed from the draught of the fume cupboard as soon as evaporation of the solvent is completed.

Three-day-old adult flies are then placed in the jars—fifty to sixty in each. The flies have previously been removed from stock jars by carbon dioxide anaesthesia and counted into test-tubes, from which they are tipped into the treated jars. No attempt is made to separate the flies into sexes for the tests (compare with the method used at East Malling Research Station). The lids of the jars are then screwed on. Each lid has a hole, through which is placed a cotton-wool plug soaked in a 5 per cent. aqueous solution of sucrose. The jars containing flies are stored on their sides at constant temperature.

Mortality counts are made between 20 and 40 hours after introducing the flies to the treated jars.

The extracts may contain substances that interfere with the toxic action of the pesticide present. For this reason, an extract of untreated material is added to the range of doses of pesticide used as a standard, in order to determine the amount present in the samples. The amount of this extract added is determined by obtaining a value for the dry weight of a known volume of the extract solution.

It has been found that the presence of interfering extractives reduces the loss of volatile insecticides during evaporation of the solvent. This phenomenon is sometimes made use of by adding unpoisoned extract to poisoned extracts that have little interfering substance present and are expected to contain a highly volatile residue.

By the procedure described above, small amounts of a wide range of pesticides may be detected. For example, 0·15 μg of dieldrin per treated bottle may be detected quantitatively.

Smaller amounts may be detected by adding a known amount of toxicant to both the sample extract and an unpoisoned extract, the difference between the two treatments being a measure of the amount of pesticide residue present.

If residue levels are particularly high and it is not necessary to make an extract in order to concentrate the toxicant present, a more simple procedure is used. The material is pulped and placed in a suitable container, and *D. melanogaster* adults are introduced into the container above the pulp. They become poisoned by contact with the pulp and, if it is a suitable food, by ingestion of the pulp. By this procedure, endrin and dieldrin have been assayed at levels of 0·3 and 0·2 p.p.m., respectively, in the pulp. There is reason to believe that Phosdrin could be assayed at a level of 0·1 p.p.m., but this has not been established.

East Malling Research Station, nr. Maidstone, Kent
Plant Protective Chemistry Section

(Mr. R. P. Tew and Miss J. M. Sillibourne)

Residue assays by a biological test have been undertaken during the past year. This was initially made necessary by a need to determine residues of endrin in blackcurrants, for which the chemical method was regarded as unsatisfactory below about 0·5 p.p.m.

Adult *D. melanogaster* are used in a method similar to that employed by "Shell" Research Ltd. Specimen tubes, 3 inches × 1 inch, form the exposure containers, and the extract solutions, after suitable "cleaning up," are evaporated in the draught of a fume cupboard.

It has been found that results are more satisfactory when only one sex of flies is used, and experiment has established optima for insect density and holding temperature. Lethal-dose (LD) values (in micrograms of poison per tube) have been determined for some of the poisons in which there is field interest at the Research Station, and the method is currently used for the bioassay of aldrin, dieldrin, Rogor and thiodan, for which LD values in the range 0·05 to 0·15 μg are encountered. When the amount of pesticide required to produce a lethal dose exceeds 0·2 to 0·3 μg (*e.g.*, with malathion, diazinon, gusathion, delnav and phenkaptone), larger samples of plant material become necessary for a suitable dosage range and more

elaborate "cleaning up" is essential to reduce the masking effect of plant extractives. Some poisons, *e.g.*, Sevin and fluoracetamide, have been found to be without toxicity to *D. melanogaster* by this test procedure.

Publication by the Research Station of experimental details and results is proposed for the near future.

Chesterford Park Research Station (Fison's Pest Control), nr. Saffron Walden, Essex

(Mr. D. T. Saggers)

At Chesterford Park the bioassay of residues is not routine. Techniques are available, but are used only when absolutely necessary, *i.e.*, when no chemical method is available or when the chemical method is unsatisfactory. Bioassay has been used for residues of endrin and Rogor.

A wingless mutant of *D. melanogaster* Meig. has been used for assaying residues of endrin and Rogor in plant material. The extract solutions for assay are evaporated on circular glass plates, 9 cm in diameter. Glass cylinders, 2 inches in diameter and 1 inch high, are coated with talc, the excess of powder is shaken off, and the cylinders are placed on the glass plates. Wingless flies are introduced into the cylinders. The flies cannot climb the talc-coated glass and so have to remain on the glass plate treated with the extract. Thirty flies are introduced into each cylinder. After exposure to the dry film on the plate for 1 hour at constant temperature and a relative humidity of 70 per cent., the flies are removed to specimen tubes containing honey-soaked food pads. Mortality counts are made after storage for 24 hours at 75° F in these tubes. Rogor at 0·01 p.p.m. has been assayed by this method.

Young *Lebistes reticulatus* (guppies), 0·5 inch long, have been used at this laboratory for residue assay. They are exposed to the extracts of material to be assayed in aqueous media. Two fish are placed in 50 ml of aerated distilled water in a 100-ml beaker, which is floated on the surface of a water bath maintained at 75° F. Under these conditions, unfed and unpoisoned fish will remain alive for at least 1 week. Pentachlorophenol and endrin have been assayed in this way at concentrations of 0·1 and 0·01 p.p.m., respectively, in the water.

The use of wingless *D. melanogaster* and guppies is preferred by this laboratory to some other methods because of the comparative ease with which they can be reared and the simplicity of the assay procedures. It is emphasised that simple techniques, so long as they work, give the most reproducible results.

Murphy Chemical Company, Wheathampstead, Herts.

(Mr. J. C. Hall)

The Murphy Chemical Company's laboratories in Wheathampstead use bioassay for residues of research compounds.

At the time the visit was made the technique employed involved use of adult *D. melanogaster* Meig. These were exposed to a dry film of an extract formed on the inside surface of the bottom of a 9-cm Petri dish by evaporation of solvent from a solution. The flies were then confined in the dish by means of glass lids. The dishes *plus* flies were stored at 70° F until mortality counts were made. Small trays containing food (sugar solution on cotton-wool) were placed in the dishes shortly after the flies. This method was adapted from that described by de Pietri-Tonelli.[1]

This technique has recently been abandoned in favour of the method used by "Shell" Research Ltd. and East Malling Research Station, which has been found superior; the control mortalities are lower, and the general manipulation of the technique is preferable to that of the method outlined above. Bioassay has so far been used only for organo-phosphorus compounds.

Hawthorndale Laboratories, (I.C.I., Ltd.), Jealott's Hill Research Station, Bracknell, Berks.

(Mr. J. F. Newman)

The Hawthorndale Laboratories of Imperial Chemical Industries Ltd. rely almost entirely on chemical analysis for the assay of insecticide residues in plant and animal materials.

Bioassay methods have been developed for use when necessary. One method has been reported[2] and involves use of newly hatched larvae of the mosquito *A. aegypti* L. These larvae are exposed to the material or extract in aqueous medium (1·5 ml) in small specimen tubes (0·5 inch × 1 inch). Those larvae unable to swim after a certain exposure time are considered as affected. The method of assessing the larvae affected is by visual observation (compare with the method in use at Rothamsted). A 50 per cent. response of the larvae may be produced by as little as 0·002 μg of parathion in each tube.

Another method involves use of an aqueous plant extract, which is spun in a centrifuge to remove suspended material and is then sprayed on to aphids. *Acyrthosiphon pisum* (pea aphid) and *Megoura viciae* (vetch aphid) have been used with success, but only relatively high concentrations of residues can be detected by this technique. Certain organo-phosphorus insecticides have been assayed in bean plants at a concentration of about 1 p.p.m.

Rothamsted Experimental Station, Harpenden, Herts.

(MR. P. H. NEEDHAM)

The method of residue bioassay at Rothamsted is similar to that used at the Hawthorndale laboratory. Second instar larvae of *A. aegypti* L. are exposed to the extract or material in aqueous medium (2 ml).

The larvae are not sorted into affected and unaffected by visual examination. They are, after exposure to the residue, placed in water in a long trough. The larvae are concentrated at one end of the trough, and a bright light is directed into this end. The normal larvae swim away from the light, and the affected ones remain at the illuminated end of the trough. Thus an entirely objective method of sorting the larvae is used.[3]

This method has been used with success for the assay of insecticide residues in poisoned honey bees. The assay levels for parathion, dieldrin, DDT and BHC are 0·02, 0·02, 0·004 and 0·006 μg per 2 ml of test medium, respectively, the figures representing 50 per cent. mortality levels.

The main disadvantage of techniques involving young mosquito larvae is that they are particularly sensitive to other extractives present in the extracts. Honey-bee extracts, for example, proved to be so toxic that they completely masked the toxicity of the insecticide residues. This means that elaborate "cleaning-up" procedures must be adopted.

Boots Pure Drug Company, Veterinary Science Division, Thurgarton, Notts.

(MR. I. R. HARRISON)

The Veterinary Science Division Research Laboratories of Boots have developed a bioassay technique for use with residues on sheep fleece. This involves use of larvae of the sheep blow-fly, which are exposed to the treated wool in a small tube containing sterile sheep serum. Concentrations of 50 p.p.m. of aldrin, BHC, dieldrin or DDT can be assayed in this way by observing the percentage mortality in 24 hours. Lower concentrations (as little as 1 p.p.m.) can be detected by observing the effect on the growth rate of the larvae.

This technique can be used for assaying insecticidal solutions. A cotton-wool plug is impregnated with the test solution, and blow-fly larvae are exposed to the cotton-wool for a 24-hour period.

The effects of DDT, BHC, dieldrin, diazinon, aldrin and many other insecticides have been compared by this method.

National Vegetable Research Station, Wellesborne, Warwicks.

(Mr. G. A. WHEATLEY AND Mr. J. A. HARDMAN)

This laboratory has developed a bioassay technique for insecticide residues in soil, but which may work equally well with residues in foodstuffs. Adult *D. melanogaster* are exposed to the extracts of the test materials in the form of a dry residue on a cotton-wool dental roll, 2 ml of extract being absorbed on each roll. While the solution evaporates, the rolls are suspended from a slowly rotating circular frame placed in an exhaust tunnel. This ensures that the residue dries evenly over the surfaces of the rolls. Syrup solution is added to the rolls, and they are hung by means of small hooks in small tubes having funnel-shaped

bases. Thirty adult male *D. melanogaster* are introduced into the tubes, which are then closed with muslin tops.

As the flies become affected, they fall to the bases of the tubes. The funnel shape guides them to the opening, which is closed by a shutter. A logarithmic "clock" mechanism opens the shutters on the tubes at logarithmically spaced time intervals. When a shutter opens, the affected flies fall on to a sticky drum, which is rotated slightly after each opening of the shutters. A record is thus obtained of the time - mortality response of the flies to the residue being tested.

The purpose of the "clock" mechanism is to obviate the necessity of making visual observations at inconvenient times. It has also been found that, with certain insecticides, the interval between records has to be small (in the region of about 10 minutes), so that it would be physically impossible to complete one record before the next had to be made. Each tube would then have a different assessment time, and a strict time sequence could not be followed.

Three-day-old *D. melanogaster* adults are used for these tests, because it has been found that there is a large variation in susceptibility during the first three days of the flies' lives.

Dieldrin at a level of 0·1 μg per cotton-wool roll gives a 50 per cent. mortality of the flies after exposure for 2 or 3 days; 0·3 μg produces the same response in about 24 hours, and 0·8 μg in about 11 hours. The exact times depend on the susceptibility of the fly culture in use.

It is possible that the sensitivity of this technique could be increased if the extracts were evaporated on to a glass surface or filter-paper. With the technique as it is at present used, the absorbing surface must be capable of holding about 1 ml of both extract and syrup solution. Unless this volume can be used, it is unlikely that crude extracts could be handled, and any concentration of the extract may lead to a loss of toxic residue during the concentration process. It is also desirable that sufficient volume of syrup solution should be used for it to remain moist throughout the entire test. This is necessary to attract the flies to the cotton-wool.

Laboratorium voor Insecticiden onderzoek, Utrecht

(Dr. D. Dresden and Dr. F. J. Oppernoorth)

The work here is mainly on the mode of action of insecticides. However, they have techniques and stocks of insects available and are occasionally called upon to do residue tests by bioassay.

Two techniques have been used. The first was a method involving use of adult *D. melanogaster* Meig. exposed to a pulp of carrots containing residues; with this, they were able to detect dieldrin at 0·5 p.p.m. and aldrin at 0·2 p.p.m. In the second method, *D. melanogaster* were exposed to a dry film of a purified extract in jars, as described by Hoskins, Witt and Erwin.[4] This method was used to determine residues in poisoned house-flies, and amounts of BHC of the order of 0·6 to 0·7 μg per exposure jar gave 50 per cent. mortality of the *D. melanogaster* after exposure to the extract for 3 hours.

During talks with Dr. Dresden of this laboratory, he suggested a most novel way of making the bioassay of residues specific. There is such a wide range of insecticide resistance by house-flies that it should be possible to identify insecticides by the particular strain of flies they do or do not kill. The maintenance of large numbers of strains of house-flies would be no easy task and would only be possible in a large and well equipped laboratory.

Keuringsdienst van Waren, Amsterdam

(Mr. H. Brunink)

Mr. Brunink's laboratory deals with market control of fruit and vegetables. The search for insecticide residues is part of this work. Bioassay is used as a qualitative test to "screen" all samples taken for insecticide analysis. Those giving a positive result in the bioassay are examined more closely by paper chromatography and chemical analysis.

The bioassay procedure involves use of *Daphnia* spp. and is described below.

The plant material is extracted with light petroleum. The sample is chopped (not macerated) and set aside in the solvent for 24 hours (1000 g of material to 1000 ml of

light petroleum). A 20-ml portion of the extract is evaporated in a 100-ml beaker. This evaporation is normally carried out at room temperature by setting the beaker aside overnight, but when greater speed is required the solvent is evaporated on a water bath until only 1 or 2 ml remain. This is evaporated by an air current at room temperature. This latter method is not preferred because of the possible loss of volatile insecticides and those unstable to heat.

The dry residue is dissolved in 0·5 ml of ethanol, and 50 to 60 ml of tap-water are then placed in the beaker. Twenty *Daphnia* are placed in the beaker by pipette, and the volume is made up to 100 ml. The mortality of the *Daphnia* is recorded at certain time intervals, and the time required to obtain a 50 per cent. mortality is recorded (LT_{50}).

When the LT_{50} is very short, and in consequence the counting time and zero time variations introduce large errors, the original extract is diluted and further bioassay is carried out. If this "screening" is carried out with care, it may be used as a quantitative determination of the insecticide residue, after this has been identified, by comparing the LT_{50} obtained with those obtained from a series of dilutions of the insecticide in question.

No useful controls are included in the tests carried out by Mr. Brunink because of the difficulty of obtaining material similar to that being tested, but not treated with insecticide. It is interesting to note here that, in Zurich, Dr. Eichenberger uses plant material from an experimental farm for his control extracts. He assumes that slight varietal differences will not make for large errors. These are probably better than no controls at all.

The sensitivity of the test is of the order shown by the figures below.

Insecticide	Phosdrin	Endothion	Parathion	Dipterex
Amount present per test beaker, μg	10	100	10	50
LT_{50}, minutes	40 to 60	40	30	120

Smaller quantities can be detected, but they give much longer LT_{50} times.

A suitable second test animal is being sought by Mr. Brunink to avoid the possibility of a residue passing undetected because of lack of sensitivity of *Daphnia* to particular insecticides. For instance, malathion and diazinon are unlikely to be detected.

Mr. Brunink is of the opinion that bioassay, as he is using it, is essential because of the enormous amount of chemical work required to search for all the possible insecticides that may or may not be present in a sample.

Farbfabriken Bayer, Pflanzenschutz Wissenschaftliche Abteilung, Leverkusen

(Dr. H. Kükenthal, Dr. H. F. Jung and Prof. G. Unterstenhöfer)

Bioassay of pesticide residues is done by Farbfabriken Bayer only when no adequate chemical or biochemical method is available. They are not only interested in organo-phosphorus insecticides, but also in all other groups of chemical compounds.

Here, as at most of the laboratories visited, there were other techniques available that would with slight changes be suitable for residue assay. Screening tests for research compounds in the public health insecticide field involved use of various residual-film tests. These were being used with susceptible insects, such as adult mosquitoes and house-flies.

Biologische Bundesanstalt für land- und Forstwirtschaft, Braunschweig Laboratorium für Zoologische Mittelprüfung

(Dr. P. Steiner and Dr. E. Mosebach)

Laboratorium für Chemische Mittelprüfung

(Dr. H. Zeumer and Dr. K. Neuhaus)

The German government laboratories have recently started work on determining insecticide residues because of a recent law relating to residue levels in foodstuffs. This law states that residues of plant-protective agents must not exceed certain fixed tolerances. These tolerances had to be established by the end of 1959. In order to determine the residues in certain crops, Dr. Steiner and Dr. Mosebach have developed a method of bioassay involving

use of a pulp of the sample containing the residue. *D. melanogaster* Meig. are exposed to the pulp of the test material in a way that gives extremely consistent results, which agree well with those from chemical determinations on the same samples (Dr. Zeumer and Dr. Neuhaus).

The bioassay procedure is described below.

Pulp (20 g) is placed in a crystallising dish (approximately 9 cm in diameter) with a filter-paper collar between the walls of the dish and the pulp to absorb excess of moisture. A cellophane cover is fastened over the dish, and a hole about 0·5 inch in diameter is made in it. *D. melanogaster* (adults) are anaesthetised for 1 minute with carbon dioxide. While anaesthetised, they are counted into specimen tubes—20 individuals per tube. Males only, between 24 and 48 hours old, are used for the tests.

When the flies start to recover from the anaesthetic, they are tipped into the crystallising dish through the hole in the cellophane cover. This hole is then closed by placing over it a piece of moistened cellophane. When the cover is dry, numerous small pin-holes are made in it to allow for air change.

The dishes containing the *D. melanogaster* are then turned upside-down on a muslin shelf and stored in this way until mortality counts are made. The counts are made in a way such as to obtain an accurate estimate of the time required to kill 50 per cent. of the flies (LT_{50}).

The figures so obtained are compared with those for known amounts of the insecticide added to a pulp from untreated produce. In this way, the amount of residue present in the test sample can be determined.

By this technique aldrin and dieldrin in carrots are being assayed. The LT_{50}, dependent on the effective material and its concentration, is usually attained within 24 hours, but with extremely low concentrations the time may be longer.

Successful assays are being made on radishes containing 0·2 to 0·9 p.p.m. of aldrin and carrots containing 0·008 to 0·7 p.p.m. of aldrin.

Further details of this technique will be given in a publication by E. Mosebach and P. Steiner, "Biological Proof of Aldrin and Dieldrin Residues in Radishes and Carrots," which will shortly appear in *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes, Brauschweig*.

Institut für Pflanzenpathologie und Pflanzenschutz, Der Georg-August Univerität, Göttingen

(Prof. Dr. W. H. Fuchs and Dr. S. Bombosch)

Dr. Bombosch has worked on a method of making bioassay specific. This has been published[5] and describes the identification of insecticides by the slope of the dosage - mortality response of *D. melanogaster* Meig. adults when exposed to a dry film of the poison in Petri dishes. Certain poisons give different changes in kill for a given change in dose. This, naturally, does not distinguish between all the known insecticides, but it does narrow the field for further investigation.

Another method is currently being investigated by a student. This seeks to identify insecticides by the picture obtained when the results of time - mortality tests are plotted graphically. This work has only recently begun and hence it is not possible to say how well it will work, but it seems to be successful for classifying the insecticides into groups.

In conjunction with the above work, the variability of the resistance to insecticides of *D. melanogaster* is being investigated. From the preliminary results, it is evident that, if great precision is required from bioassay tests, the rearing of *Drosophila* is not as simple as it appears.

Bundesanstalt für Qualitätsforschung Pflanzlicher Erzengnise Geisenheim/reingau

(Prof. W. Schuphan and Dr. W. Weinmann)

This laboratory deals with the quality of fruit and vegetables from the nutritional point of view and in relation to other special applications, such as industrial uses. The detection and determination of insecticide residues has recently become a part of its work.

The bioassay method used has been described[6] and involves use of adult *D. melanogaster* Meig. exposed to dry films of the extracts deposited on the interior surfaces of Petri dishes. Tests have been carried out on a wide range of fruits and vegetables contaminated with a wide variety of insecticides. From these tests it would appear that the method is very sensitive. Parathion was determined in apples at a concentration as low as 0·06 p.p.m.

A rather complex extraction and "cleaning-up" procedure is used and is described in detail in the paper mentioned above.

Chemische Laboratorium der Stadt Zurich, Insectizid-abteilung, Zurich

(Dr. J. Eichenberger)

This laboratory was most interesting to visit because great reliance was placed on bioassay. Although Dr. Eichenberger has recently re-equipped his laboratory to make more use of chemical and chromatographic methods of analysis, he is still of the opinion that bioassay is essential in the search for insecticide residues in market produce—this being his main line of work. As far as could be ascertained, the bulk of the work is bioassay; the chemical and biochemical procedures are ancillary to it.

Several bioassay methods are used. A mosquito-larva (*A. aegypti* L.) test is used, which is similar to that described by Burchfield, Hilchey and Storrs,[3] although it differs in detail and was developed independently.[7] The reaction to light of the larvae is utilised to distinguish between those poisoned and those unaffected by the residue in an extract.

The light-irritation test with larvae of *A. aegypti* will assay the insecticides shown in Table I at the levels indicated.

D. melanogaster adults are used in a direct method, in which they are exposed to a pulp of the test material, and in a test involving a dry deposit of an extract in small bottles, the *D. melanogaster* being confined in the bottles with the deposits. House-flies are used in the direct method as are the *D. melanogaster* and in another method in which an extract of the test material is added to their food.

Table I

Levels of insecticides assayed by larvae of *A. aegypti* L.

The LT_{50} values shown are typical of those obtained under the working conditions

Insecticide	Concentration in test solution, p.p.m.	LT_{50} minutes	Notice
Parathion	0·001*	1380	Under limit
	0·005*	446	
	0·01*	225	
	0·01†	205	
	0·08†	46	
Diazinon	0·02*	130	Under limit
	0·10*	75	
DDT	0·005†	72	
	0·08†	22	
	0·004*	255	
	0·01*	117	
	0·1*	30	
Lindane	0·005†	165	
	0·05†	19	
Endrin	0·005†	240	
	0·05†	38	
Aldrin	0·01†	212	
	0·08†	92	
Dieldrin	0·005†	165	
	0·05†	46	
Heptachlor	0·08†	98	
Toxaphene	0·08†	123	

* Active substance in extract.
† Active substance in pure solution.

From the results of these tests it is claimed that only a lead can be obtained as to the identity of the group to which a residual insecticide belongs. This is done by observing the symptoms of intoxication of the test organism and by comparing the relative results from various bioassays. These methods, which depend on observations of the behaviour of the test organisms during a test, would be difficult to adopt as standard procedures. They depend to a large extent on the individual opinion of the observer. For this reason, positive identification of the active substance is made by paper-chromatographic or colorimetric reactions.

Dr. Eichenberger intends to use another method of separating extracts into fractions containing different groups of insecticides. This is to treat the extract in a way such as to obtain fractions containing insecticides resistant to acid or alkali or volatile by steam-distillation. Chromatographic separation will also be used. These fractions will then be tested biologically. By this method and the one outlined above, it is hoped to identify and determine insecticide residues.

It is interesting to note here that this laboratory has attempted to use *Artemia salina* (brine shrimp) for bioassay. The method was abandoned for several reasons. It was very laborious to rear the shrimps to the adult stage, the small *A. salina* (nauplii) are not active enough to allow easy macroscopical assessment of the effect of insecticides, and the saline medium was found to be unsuitable for testing certain extracts.

J. R. Geigy A. G., Basle

(Mr. R. Wyniger)

In the Plant Protection Laboratories of Geigy A.G., all samples for residue determination are subjected to chemical or, if no adequate sensitive analytical method is available (especially for new compounds), bioassay tests.

Daphnia pulex used to be the bioassay test material, but its use has now been discontinued because of great sensitivity to slight changes in the rearing conditions, *e.g.*, changes in the pH of the water and in the amount of illumination. This made *D. pulex* an unreliable test organism. The larvae of *A. aegypti* L. are now used with success, although they are not so sensitive to certain insecticides. The difference is, however, relatively small. *D. pulex* are twice as sensitive to parathion as are *A. aegypti* larvae.

The larvae are exposed to extracts of the test material in water in small shallow dishes. These are inspected at certain time intervals, and counts of the affected larvae are made. From these figures an estimate of the time required to kill 50 per cent. of the larvae is obtained. This is compared with the kills from standard dilutions of insecticides, and the amount of insecticide in the test material is determined.

The counts of the affected larvae are made by purely visual observation of the larvae in each dish. This is in contrast to the technique used by Dr. Eichenberger and at Rothamsted, in which the affected larvae are sorted from the unaffected by their ability to respond to the stimulus of strong illumination. It is possible that the latter technique increases the sensitivity of the mosquito-larva test, since the inhibition of the response to light is caused by lower concentrations of some insecticides than are required to kill.

The mosquito-larva test is sensitive to 0·01 and 0·1 p.p.m. of parathion and endrin, respectively, in 20 ml of water, these concentrations giving 50 per cent. response in 1 hour.

This laboratory also uses another technique, which I did not see during my visit. Adult house-flies receive a drop of water containing an extract of the test material. By this method, assays may be made of dieldrin and lindane as low as 0·5 μg per replicate test.

From the preceding account of visits to certain laboratories in Europe, it can be seen that a variety of techniques involving a selection of test organisms has been used for the detection and determination of pesticide residues in foodstuffs. The following sections contain an account of the more important literature relating to the bioassay of pesticide residues.

INSECTICIDE RESIDUES

The most commonly favoured test organisms for detecting residues of insecticides are adult *D. melanogaster* (vinegar-fly), adult *M. domestica* (house-fly) and larvae *A. aegypti* (yellow fever mosquito), although it will be shown that others have been used to a limited extent.

The techniques in which the test organisms have been used may be divided into three types.

(*i*) The direct methods, in which the test organisms are confined over a pulp of the residue-containing material or the material is fed in some way to the individuals.

(*ii*) The film methods, in which the test organism is exposed to the residue after evaporation of the solvent from an extract of the sample to be assayed.

(*iii*) The aqueous methods, in which the residue, after extraction from the sample, is added to water containing some species of aquatic organism.

DIRECT METHODS—

Adult *D. melanogaster*[8 to 13] have been used most frequently with these methods; adult *M. domestica* have been used to a lesser extent.[14,15]

These tests are generally of relatively low sensitivity compared with most techniques for residue bioassay.[16] Gyrisco and his co-workers[12] detected 0·1 p.p.m. of aldrin and dieldrin in fruit pulp. Pankaskie and Sun[11] claim the same order of sensitivity with plant materials generally. Fisher and Smallman[10] state that valid determinations were made of aldrin in pumpkin at concentrations as low as 0·05 p.p.m., the flies being exposed to the pulp for 24 hours.

The maximum sensitivity of this method is usually only achieved by long exposures to the pulp. Thus Mosebach and Steiner (see report on visit to Biologische Bundesanstalt für Land- und Forstwirtschaft, Braunschweig) found that, although 0·008 p.p.m. of aldrin could be determined in carrot pulp, the exposure time required to kill 50 per cent. of the flies may be as long as 2 or 3 days.

Fisher and Smallman[10] state that their pulp method is a feeding method. When flies are exposed to a pulp, the toxic action of the residue may be exerted by contact of the flies with the pulp or by the ingestion of the pulp if it is an attractive food.

A direct-feeding method has been described,[14] in which milk was absorbed on cotton-wool, and adult house-flies were then exposed to this. Concentrations of 0·1 p.p.m. of dieldrin or aldrin and 0·2 p.p.m. of lindane were detected in milk by this technique.

Frawley, Laug and Fitzhugh[15] described a direct-feeding method in which an ether extract of the sample to be tested was evaporated over sugar. The sugar was then dissolved in water, and the solution was fed *ad libitum* to *M. domestica* for 24 hours. This technique was able to detect 3 μg of parathion, 5 μg of lindane and 15 μg of DDT in a variety of fruits and vegetables.

FILM METHODS—

These methods, like the direct techniques, have been used with adult *D. melanogaster* and *M. domestica*.

The film may be formed on the inside surface of small glass jars,[4,17,18,19] small tubes,[20] flasks[21,22] or Petri dishes [1,5,6] or on filter-papers.[23]

Sun and Sun[24] have assayed as little as 0·4 μg of aldrin and dieldrin per exposure vessel with *M. domestica*. This represents 0·1 p.p.m. in a 100-g sample of contaminated material. Klein and his co-workers determined 0·1 to 0·5 p.p.m. of endrin in leafy vegetables by using *M. domestica* in a dry-film bioassay.[21] Hoskins, Witt and Erwin used the same insect for assaying lindane at 0·8 μg per exposure bottle.[4]

Some workers have used a dry film,[19,20,24,25,26] but others claim that the addition of small amounts of an oily substance to the film increases the pick-up of the toxic residue by the flies[4] and improves the distribution of the film on the surface.[4,17,22,27,28] These oily substances may be light mineral oils or vegetable oils, such as olive oil or corn oil. The presence of oily substances reduces the losses of volatile pesticides during evaporation of the solvent,[29] but may to some extent mask the toxicity of the film of residue.[18,21,30]

The process of extracting the toxic residue from a sample may also extract other materials present that exhibit a toxic action to the test organism, particularly if the extract is concentrated to increase the sensitivity of the bioassay.[17,22,28,30,31] Steps have to be taken to remove the interfering substances from the extracts before bioassay.

With *D. melanogaster*, Kastings and Harcourt[32] were able to assay 0·06 p.p.m. of parathion n cauliflower. With the same test insect, lindane and aldrin have been assayed at 0·8 and 0·05 μg, respectively, per exposure vessel.[19] These figures are stated to be one-fifth of the dose required to kill 50 per cent. of the flies.

AQUEOUS METHODS—

This group of techniques has mainly been used with the larvae of *A. aegypti*[2,3,7,33 to 41] and represents the most sensitive bioassay method available. Newman was able to determine parathion at 0·002 μg in each replicate of an assay.[2]

Other test organisms have been utilised for these methods; among them are species of *Daphnia* (water flea),[42,43,44,45] *Gammarus pulex* (freshwater shrimp),[46] *Artemia salina* (brine shrimp)[47,48,49,50] and certain fish. Pagan and Hageman[51] and Pagan[52] used guppies (*Lebistes reticulatus*), and goldfish have also been used.[53,54]

Direct methods with A. aegypti—Starnes[40] used, as well as a method involving an extract, a form of direct method with fourth instar *A. aegypti* larvae. Macerated diluted plant material (potato foliage) was placed in the water containing the larvae. Some success was gained with the method, but the plant material showed toxicity to the larvae and suppressed to some extent the toxicity of the insecticidal residue.

A direct method was used by Bushland[36] in an attempt to determine residues of DDT, TDE, methoxychlor, chlordane, toxaphene and lindane in butterfat. Fourth instar *A. aegypti* larvae were immersed in a 4 per cent. emulsion of butterfat. Although the larvae survived for at least 24 hours in the emulsion, they failed to be killed by the addition of 1 p.p.m. of the toxicants. So great was the effect of the interfering substances that concentrations well in excess of a lethal dose in water were not detected.

Extracts of the samples to be tested have been used in most techniques involving aqueous methods of bioassay.[2,7,35,39,41,55,56,57]

Extract methods with A. aegypti—*A. aegypti* larvae have been used by some workers with an extract in relatively large volumes of water.[40,41] This meant that large samples had to be used in order to obtain sufficient residue to produce a toxic effect. Newman[2] increased the sensitivity of the method with *A. aegypti* by using first instar larvae and exposing them to the extract in only 1·5 ml of water, so that the total amount of toxicant required was small. A visual examination was made, and the absence of activity of the larvae was the effect recorded. By this method a 50 per cent. response of the larvae was obtained to 0·002 μg of parathion in each 1·5 ml of water.

Various workers[3,7,35] have improved the means for determining whether or not a mosquito larva is affected by a toxicant. Early instars of *A. aegypti* are difficult to observe with the naked eye. Consequently, use has been made of the fact that they are negatively phototactic, *i.e.*, they move away from light. This response to light is inhibited by very low concentrations of poison. Thus, by using a suitable apparatus, normal and affected larvae may be distinguished easily and objectively. Relatively large volumes of water were used. Burchfield and his co-workers[3] used 50 ml for each replicate of larvae, so that the method is not as sensitive as that described by Newman.[2] A 50 per cent. response was obtained in a reasonable time to lindane at 0·01 p.p.m., parathion at 0·02 p.p.m., aldrin at 0·005 p.p.m. and DDT at 0·005 p.p.m.

Interference—Even when extracts are used with mosquito larvae, interference from extractives is troublesome. It has been found that the extractives of the honey bee are so toxic to *A. aegypti* larvae that, unless some cleaning procedure is used, bioassay by this method is impossible.[55,57] It has been found necessary to remove interfering extractives from extracts of other materials.[35,41]

Other organisms—Species of *Daphnia* have been used for the bioassay of pesticide residues. Anderson[43] assessed the toxicity of industrial wastes by their use and later reported them to be extremely sensitive to DDT.[58] He also described[59] *D. magna* as being more sensitive indicators of water pollution than are fish. Kocher and his co-workers[42] used *D. pulex* to assay insecticide residues in fruits and vegetables. A description of a technique for bioassay has been given by Pfaff.[44] A value for the 50 per cent. response of *D. pulex* to parathion has been given as 0·5 p.p.m. in each test container,[42] this being a mean value obtained from various times of exposure. With longer times, in the region of 8 hours, 0·05 p.p.m. could readily be detected. The parathion was in an extract of cherries.

The anostracan *Artemia salina* has been used as bioassay material for residue determination. The first mention of its use was an anonymous contribution in 1955.[47] Since then, Tarpley[48] and others[49,50] have advocated the use of *A. salina* in bioassay. Various instars have been tested in order to find the one most suitable. Tarpley found that fifth instar *A. salina* gave a 50 per cent. response to parathion at a concentration in the test vessel of

0·320 p.p.m. after an exposure of 5 hours. The conclusion reached was that they are not so sensitive as are the larvae of *Aedes aegypti*. These results were obtained with dilutions of pure insecticide and not with extracts. The interference from extractives does not appear to have been investigated.

Fish have been used to assay residues in an aqueous-type test. Pagan[52] selected *Lebistes reticulatus* (guppies) for a toxicological assay of derris and lonchocarpus roots. Pagan and Hageman[51] used them to detect small amounts of DDT in plant materials and milk. The LD_{50} of DDT was approximately 0·04 p.p.m. after an exposure of 24 hours. The fat in milk was found to interfere with the toxic action of the DDT and was itself toxic after long exposures. *Carrasius auratus* (goldfish) have been used for residue assay.[53,54] These were used to determine a number of chlorinated hydrocarbon insecticides in plant and animal materials. A 100 per cent. reaction (convulsions and loss of equilibrium) of the fish was obtained at the following concentrations (micrograms per 250 ml of water): lindane, 50; DDT, dieldrin, methoxychlor or toxaphene, 100; Dilan or heptachlor, 200; aldrin or chlordane, 500.

MICRO-ORGANISMS—

A wide range of bacteria, yeasts and protozoa have been investigated to ascertain their suitability for insecticide bioassay. The results with DDT and lindane were negative. Parathion, however, affected the behaviour of *Paramecium multimicromeleatum* at a concentration of 1 p.p.m.[60]

FUNGICIDE RESIDUES

The bioassay in foodstuffs of residues of other pesticides, such as fungicides and herbicides, and the use of organisms other than insects have received little attention. Several methods of detecting the presence of small amounts of fungicides have been described, although few of them have been developed for the purpose under consideration here.

A method for detecting small amounts of fungicide on seeds has been described by Mead.[61] The seeds were placed on agar plates previously seeded with a suspension of spores of *Helminthosporium sativum*, and growth of the fungus was inhibited by the fungicide on the seeds. No details about sensitivity were given. Leben and Keitt[62] detected microgram amounts of tetramethylthiuramdisulphide (TMTD) on corn seeds by absorbing an ethanol extract of the seeds on blotting-paper discs. The discs were placed on agar plates seeded with *Glomerella cingulata*.

These techniques depend on the diffusion of the fungicide or other toxic substance into the agar. A zone of inhibition of the test organism is formed in this way. Leben and Keitt,[63] Thornberry[64] and Arny and Leben[65,66] have all used this agar-plate diffusion method for the detection and determination of small amounts of fungicide. A similar method has been used for determining antibiotic residues in preserved fish, the test organism being *Staphylococcus aureus*.[67]

The translocated fungicide 2-pyridinethiol-1-oxide has been detected in cucumber seedlings by the inhibited germination of spores of *Monilinia fructicola*. The samples were homogenised and spun in a centrifuge, the supernatant extract being used for the test.[68] Angelotti and his co-workers[60] investigated the possible detection of fungicides by a wide range of micro-organisms, including bacteria, yeasts and protozoa. The fungicides nabam and zineb were tested against the micro-organisms, and various techniques were used, depending on the test organism. In one, a turbidimetric method, the fungicides were added to inoculated nutrient broths and the turbidity of the resulting culture was taken as an indication of the growth of the yeast or bacterium; a decrease in turbidity indicated inhibition of growth. The assay-disc method already described was used with some of the bacteria. Lastly, the effect of the pesticides on the behaviour of individuals in cultures of free-swimming protozoa was observed.

The results from these experiments were largely negative. A graded response was, however, given to nabam and zineb by *Micrococcus pyogenes* var. *epidermis*.

Actidione (*cyclo*heximide) has been detected quantitatively in cherries by a bioassay involving use of *Saccharomyces pastorinus*.[69] Extraction into chloroform and subsequent concentration of the extract were necessary for concentrations below 1·5 p.p.m. in the fruit. Aqueous suspensions of the extract were added to plate cultures of the fungus. A concentration of 0·04 p.p.m. could be detected in the fruit by this method.

HERBICIDE RESIDUES

During this investigation no literature has been found relating to the bioassay of residues of herbicides in foodstuffs, but several methods for determining herbicidal action showed high sensitivity and might well be of use for determining residues.[70 to 78] The sensitivities of these methods have been discussed by Dewey[16] in a review of techniques for the bioassay of pesticide residues.

In addition to these methods, two procedures have been described in which the inhibition of growth of the primary roots of germinating cucumber seeds[79] and corn seeds[80] has been used as a measure of the concentration of 2:4-D present in an aqueous solution; a sensitivity of 0·005 p.p.m. was attained.[79]

Zimmerman and his co-workers[81] described a method of bioassay for residues of 2:4-D, but not in foodstuffs. The technique, which depends on the modification and curvature of the leaves and stems of cotton and tomato plants, was used for detecting the herbicide as a contaminant of other pesticides. The modification of the leaves was the most sensitive response and determined down to 1·0 p.p.m. of 2:4-D.

GENERAL CONSIDERATIONS

Quantitative bioassay is generally based on a time - mortality or a dosage - mortality response of the test organisms. The statistical methods involved have been reviewed by Sun[82] and Dewey.[16] There are, however, certain general conditions that it is essential to bear in mind when bioassay techniques are used. There are many factors that can affect the value of the results obtained from tests involving biological material. These factors fall into two groups, those relating to the manner in which the bioassay is carried out and those affecting the insects used.

Potter and Way[83] have reviewed the factors affecting the results of precision spraying techniques for the laboratory assessment of insecticides. Many of these may also apply to bioassay tests, which involve other living organisms than insects. Here will be considered these factors as they have been found by some workers to affect the performance of bioassays of pesticide residues.

Rearing of test species—

It is important for bioassay that the test organisms should be reared under carefully controlled conditions. Many workers have found[83] that certain factors relating to the rearing conditions and the storage of insects before use in insecticidal tests can affect their susceptibility to the toxicants used. Nutrition, humidity, temperature and illumination are among these factors. If accurate and consistent results are required from bioassay procedures, it is important that the effects of these factors on the test organism should be understood, and, if possible, their variation throughout a series of tests should be avoided.

Many workers have described rearing techniques for the three test insects frequently used in bioassay (*D. melanogaster*,[10,25,84] *M. domestica*[85 to 90] and *A. aegypti*[91,92,93,94]) and have studied the conditions most suitable for successful maintenance of cultures.

The rearing of the crustaceans *Daphnia* spp. and *A. salina* has received less attention. Seume[95] and Anderson[58] have described methods for rearing *D. magna*, and Sun[82] states that rearing is simple, bacteria or their equivalent being all that is required for food. The rearing of *A. salina* is unnecessary, since the eggs may be purchased at most pet-stores. Tarpley[48] and Michael and his co-workers[49,50] described methods by which adults and younger stages may be obtained from the eggs.

Age and sex of insects—

The age at which test insects are used in bioassay has been found to affect the results obtained. Burchfield and his co-workers[93] have shown that the various instars of the larvae of *A. aegypti* vary in their susceptibility and that certain instars show a wide variation due to age within the instar. If the age used is not carefully controlled, variable and inconsistent results may be obtained from the bioassays. Fisher and Smallman[10] drew attention to inconsistency of results in connection with the age of *D. melanogaster* used in the pulp method of bioassay.

The sex of the insects used in bioassay determinations has also been found to affect the results; male *D. melanogaster* were found to be more susceptible to toxicants than were

females.[10,84] To obtain the greatest precision one sex only was used. Fisher and Smallman found that uniformity of response could be obtained by using equal numbers of each sex.[10] This uniformity was destroyed when the proportion of either sex was increased.

DESIGN OF TEST—

It is of importance to design the bioassay in a way such that it is capable of giving the information required. To this end, adequate control tests both with and without added toxicant should be included. These controls should demonstrate the effects of interfering substances, toxicity from solvents and the handling of the test organisms.

The treatments given to the organisms in the bioassay should be replicated sufficiently and enough individuals should be used to ensure the validity of the results obtained.

It has been found that, when very small amounts of toxicant are involved, there is an optimum number of individuals that can be used in each replicate.[10] If too many are used, each one may only be able to pick up a sub-lethal dose.

It is important to ensure that the amount of toxic residue to which the test organism is exposed is reproducible, otherwise there may be large variations between the replicates in a test, which will make the results unreliable. In this connection, the duration of exposure to the residue must be carefully controlled if reliable quantitative results are required.[22,28]

USE OF ANAESTHETICS—

To facilitate the handling of the test insects used in bioassay, anaesthesia is often applied, and it has been found that its practice before an assay can affect the results. Edwards and his co-workers[27] found that results were erratic when ether or carbon dioxide was used as anaesthetic for *D. melanogaster* to facilitate counting and sorting into sexes. Residues of aldrin and lindane were being assayed in soils by a film method.

SPECIFICITY OF INSECTS—

It is well known that insecticides do not affect all species of insects equally. In my experience, *D. melanogaster* and *A. aegypti* are relatively insensitive to DDT and DNOC, respectively. It is important to use more than one species of test organism in order to ensure that a negative result is caused by lack of a toxic residue. This is particularly important in assays of "unknown" residues.

PHYSICAL STATE OF POISON—

In the assay of residues of lindane by a film method involving use of house-flies, Hoskins, Schiller and Erwin[96] found that no two test containers had identical size or distribution of crystals of the poison. The flies picked up different amounts of the crystals, which caused the mortality results to be unreliable. The addition of a small amount of light spray oil to the film prevented crystal formation and assisted in the production of a uniform film. Hoskins, Witt and Erwin[4] discuss other problems arising from varying the physical states of deposits in film methods of bioassay.

AMBIENT CONDITIONS—

These comprise the temperature, humidity and illumination under which the bioassay is carried out. It is important that the effects should be allowed for and, when consistent results are required, these conditions should be standardised.[20,27]

A comprehensive review of the factors affecting the use of micro-organisms in the agar diffusion and turbidimetric methods of assaying fungicides and other toxic substances for which these methods are suitable has been given by Harris.[97]

IDENTIFICATION OF POISONS

Various attempts have been made to achieve specificity in bioassay tests in order to be able to identify as well as determine the poison present. The only purely biological methods of identifying toxicants that have been described are by their effect on the behaviour of the test organism before death and by differences in susceptibility.

Burchfield and his co-workers[93] found that DDT and parathion could be identified by their effect on the behaviour of the larvae of *A. aegypti* exposed to strong illumination. A difference has been found in the inhibition of the response of the larvae to light of different wavelengths after poisoning by certain insecticides. It has been suggested that this response may be used to discriminate between certain insecticidal residues.[37]

Pfaff[44] was able to distinguish between chlorinated hydrocarbon and organo-phosphorus insecticides by the movements of *Daphnia* spp. after poisoning. DDT and chlordane have been identified by differences in their toxicity to *Macrocentrus ancylivorus* (oriental fruit-moth parasite).[23] Bombosch[5] was able to separate a number of chlorinated hydrocarbon insecticides into groups by the susceptibility to them of *D. melanogaster* and *Calandra granaria* (grain weevil). As has been mentioned earlier in this report, a method of obtaining specificity in bioassay has been suggested by Dresden, *M. domestica* resistant to certain insecticides being used.

Identification by the above methods is difficult when mixtures of pesticides are present in a sample. Fleming, Coles and Maines[23] overcame this by taking advantage of the difference in volatility of DDT and chlordane.

Identification and determination of residues and mixtures of residues is most conveniently done by a combination of bioassay and chromatographic or chemical methods. The residues may be subjected to chromatography or treated by chemical methods affecting the stability of the pesticide present in such a way that fractions are obtained that may contain certain pesticides or groups of pesticides if present. These fractions may then be subjected to a bioassay test.[21,55,82]

CONCLUSIONS

The choice of suitable techniques for the bioassay of pesticide residues is not easy.

The simplest methods are the direct ones, in which a pulp of the sample to be assayed is used. No extraction and subsequent cleaning up is required. These methods are, however, regarded as being less sensitive than others, unless inconveniently long exposure times are given (see report on visit to Biologische Bundesanstalt für Land- und Forstwirtschaft, Braunschweig). In the presence of strongly masking substances direct methods may even be useless. Hartzell[38] found that a concentration of BHC in processed peaches that gave 100 per cent. response with *A. aegypti* larvae was non-toxic in processed peas.

The methods in which extracts are used can be more sensitive than the direct methods. This increase in sensitivity is achieved by concentrating the extract obtained from a large sample. This process also concentrates the interfering substances, which can mask the toxicity of the residue or may themselves be toxic to the test organism. Thus, if extraction methods are used, complex procedures for cleaning up the extracts will generally be necessary.

There is evidence that the techniques suitable for fungicides are not applicable to insecticides and *vice versa*. Special techniques will have to be used for fungicides and herbicides.

The choice of technique is primarily one of sensitivity, whatever pesticide is being assayed.

Secondly, a test organism must be chosen. This choice will be limited to some extent by the assay method and the sensitivity required. Further limitations will be imposed by the facilities available for rearing the test organisms. It has already been stated that rearing, particularly of insects and crustaceans, has to be done under carefully controlled conditions. For most organisms, this rearing has to be continuous. The eggs of *A. aegypti* can, however, be stored for at least 1 month, which permits rearing to be on a batch basis. The eggs of *A. salina* may be purchased, and, as pointed out earlier, this crustacean need not be reared at all.

Female *A. aegypti* require a blood meal before eggs are produced. This necessitates the added complication of providing guinea pigs or other suitable animals for the purpose. Knierim and his co-workers[98] experimented with feeding the females on preserved blood, but I have tried this procedure without success.

M. domestica is a larger insect and is easier to handle, but it requires more space for rearing than does *D. melanogaster*.

D. magna and *D. pulex* require only a small space for rearing,[58] as does another test organism for use with the aqueous type of bioassay, *L. reticulatus* (guppy); the last-named is also easy to handle.[52]

D. melanogaster, as has already been stated, appears to be the most commonly used insect for the bioassay of insecticide residues in foodstuffs. It would appear also to be the most suitable insect for public analysts to use, as it can be reared in a small space. A small cabinet supplying the necessary rearing conditions is all that is required to house the culture jars. It has the further advantage of being suitable both for the direct method of bioassay and for the techniques involving extracts deposited as films.

It has been pointed out in this report that the use of more than one test organism is desirable. If *D. melanogaster* is being used as the first test insect, then *M. domestica* could be employed as the second, as it is suitable for the same bioassay methods.

There are probably many advantages to be gained from using two insects not so closely related in different techniques. If this is so, then the second organism could be *A. salina*, one of the species of *Daphnia* or *L. reticulatus* used in an aqueous type of bioassay. It may be satisfactory to use the larvae of *A. aegypti*, as a different stage in the life cycle is employed, although it is a dipterous insect like *D. melanogaster* and *M. domestica*.

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The Separation and Determination of Sugar Phosphates, with Particular Reference to Extracts of Fish Tissue

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A procedure is described for the quantitative separation of hexose and pentose phosphates present in acid extracts of tissues. The extract is neutralised to pH 7·4 and applied to a strongly basic anion-exchange resin in the chloride form. Elution is carried out by a single gradient of decreasing tetraborate and increasing acid and chloride concentrations. The phosphates are evaluated by standard colorimetric procedures.

THE comparatively recent application of anion-exchange chromatography to the resolution of complex mixtures of sugar phosphates has produced analytical methods superior in most respects to the earlier schemes involving precipitation by barium and alcohol. We report here a simple and rapid method for separating the phosphate esters of pentoses and hexoses. These compounds are important intermediary metabolites in living tissues. In foods, they are known to be potent intermediates in the series of reactions leading to non-enzymic "browning."[1]

The sugar phosphates are separated by quantitative gradient elution from an anion-exchange resin. This procedure has an advantage over older stepwise-elution procedures[2 to 8] in that continuous attendance on the apparatus is not required. It effects an improved separation of certain tissue components in comparison with earlier gradient systems,[3,9] and the basis of the gradient used is different.

Experimental

Sugar phosphates may be grouped into two classes—those that are acid-labile (the aldose 1-phosphates) and those that are not. Consequently, too low a pH was to be avoided during the elution of glucose 1-phosphate and ribose 1-phosphate. On the other hand, in order to minimise alterations to sugar moieties by enolisation, it was thought advisable not to let the pH rise above 9 at any stage.

It was found that, when a column of the type described below was used, the sugar phosphates in an extract of 20 to 25 g of fish muscle could be retained by strongly basic resin in the chloride form at pH values between 6·5 and 8·5. Extracts of tissue contain fairly high concentrations of orthophosphate and chloride, and, by comparison with a prepared mixture of the pure compounds virtually free from contaminating anions, the presence of these additional anions decreased the amounts of sugar phosphate that could be exchanged on to the resin.

Fig. 1 shows the change in pH found during elution with the solutions described under "Method." Comparison of this with Fig. 2 shows that the acid-labile glucose 1-phosphate and ribose 1-phosphate were eluted from the resin before the conditions became too acid. To reduce still further the possible break-down of these compounds on the resin, the complete column assembly, including the fraction collector, was placed in a cold room at 1° to 2° C.

A high concentration of tetraborate was required in the initial stages of elution to effect the separation of 1- and 6-phosphates. This was not so important at a later stage of the elution, when the aim was to remove from the resin compounds that had formed complexes with tetraborate and were fairly firmly attached to the resin. A decreasing gradient of tetraborate concentration was used to allow maximum binding at the beginning of the elution; to prevent excessive hold-back of bonded compounds, an increasing gradient of chloride and acid concentrations was devised.

A certain delay in the appearance of glucose 1-phosphate (the first compound of interest) in the effluent was found to be unavoidable. Attempts to reduce this by altering the relative concentrations of salts in the solutions in the mixing vessel and reservoir adversely affected the resolution obtained. Consequently, the first thirty or so fractions can be safely discarded when carrying out separations of the sugar phosphates in extracts of tissue. One compound was eluted in this region when prepared mixtures were separated; this peak was found to correspond to a so far unidentified impurity present in the sample of fructose 6-phosphate.

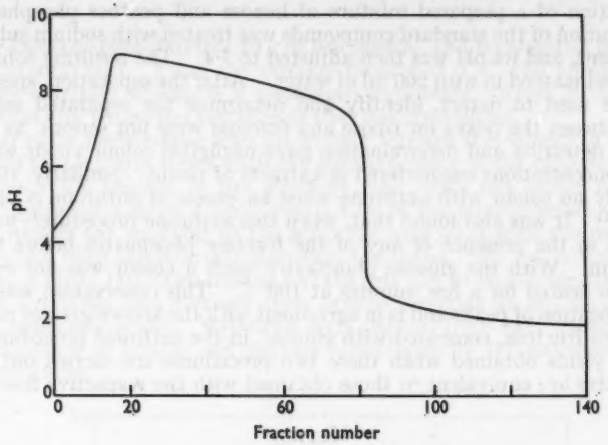

Fig. 1. Change in pH of effluent from column (53 cm × 1·8 cm) of Dowex 1–8x (chloride), 200 to 400 mesh, during elution by a gradient of 0·17 *M* potassium chloride and 0·017 *N* hydrochloric acid into 1 litre of 0·01 *M* sodium tetraborate and 0·0484 *M* chloride

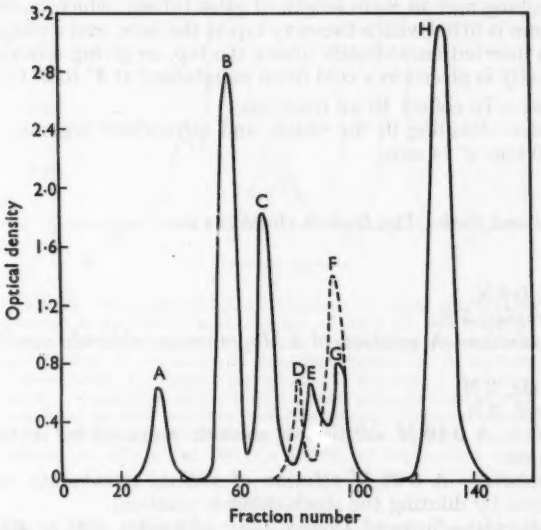

Fig. 2. Chromatographic separation of prepared mixture of sugar phosphates: A, unidentified impurity; B, glucose 1-phosphate (20 μmoles); C, glucose 6-phosphate (13 μmoles); D, ribose 1-phosphate (7 μmoles); E, fructose 1-phosphate (5 μmoles); F, ribose 5-phosphate (16 μmoles); G, fructose 6-phosphate (10 μmoles); H, fructose 1:6-diphosphate (25 μmoles). Conditions of elution as for Fig. 1; 10-ml fractions; 2-ml aliquots treated with anthrone reagent solution, and optical densities measured in 4-cm cells at 620 mμ (full-line curve); 2-ml aliquots treated with orcinol reagent solution, and optical densities measured in 1-cm cells at 670 mμ (broken-line curve)

The separation of a prepared mixture of hexose and pentose phosphates is shown in Fig. 2. The solution of the standard compounds was treated with sodium sulphate to remove the barium present, and its pH was then adjusted to 7·4. The resulting solution was run on to the column and washed in with 200 ml of water. After the separation, specific colorimetric procedures were used to detect, identify and determine the separated sugar phosphates. The overlaps between the peaks for ribose and fructose were not serious, as the orcinol procedure used for detection and determination gave negligible colour yields with hexose phosphates at the concentrations encountered in extracts of tissue. Similarly, ribose phosphates produce virtually no colour with anthrone when an excess of anthrone is present, as in the method used.[10,11] It was also found that, when this anthrone procedure[11] was used, a green colour appeared in the presence of any of the fructose phosphates before the digestion at 100° C was begun. With the glucose phosphates, such a colour was not evident until the sample had been heated for a few minutes at 100° C. This observation was found to be of use in the identification of peaks and is in agreement with the known greater rapidity of colour development with fructose, compared with glucose, in the anthrone procedure.[11]

The colour yields obtained when these two procedures are carried out on hexose and pentose phosphates are equivalent to those obtained with the respective free sugars.[12,13]

Method

Apparatus—

Ion-exchange column assembly—A glass separating funnel (the reservoir) of capacity at least 2 litres is connected through an air-tight joint to the top of a conical 1-litre mixing vessel fitted near the base with a right-angled side-arm (0·5 cm × 10 cm). The mixing vessel contains a glass-sheathed paddle and is placed on a small magnetic-stirrer motor. The side-arm of the mixing vessel is connected to the top of a glass column (70 cm × 1·8 cm) by a 50-cm length of flexible tubing and an 8-cm length of glass tubing, which passes through a rubber stopper. The column is fitted with a two-way tap at the base, and a plug of glass-wool, which retains the resin, is inserted immediately above the tap, so giving a minimum of dead space. The complete assembly is placed in a cold room maintained at 1° to 2° C.

Fraction collector—To collect 10-ml fractions.
Spectrophotometer—Reading in the visible and ultra-violet regions.
Test-tubes—150 mm × 14 mm.
Homogeniser.
pH Meter.
Buchner funnel and flask—The flask is chilled in ice.

Reagents—

Perchloric acid, 0·6 N.
Potassium hydroxide, 5 N.
Stock chloride solution—A solution of 2 *M* potassium chloride containing 0·2 *N* hydrochloric acid.
Sodium hydroxide, 2 N.
Hydrochloric acid, 2 N.
Reservoir solution—A 0·19 *M* solution of chloride prepared by requisite dilution of the stock chloride solution.
Mixing-vessel solution—A 0·01 *M* solution of sodium tetraborate containing 0·0484 *M* chloride (also prepared by diluting the stock chloride solution).
Dowex 1–8x (*chloride*)—Suspend Dowex 1–8x (chloride), 200 to 400 mesh (obtainable from Howe & Co. Ltd., 46 Pembridge Road, London, W.11), in water, and pour the slurry into the detached column until a sedimented bed 53 cm deep is obtained. Wash the resin first with 2 *N* sodium hydroxide until the effluent is almost free from chloride ions, then with 500 ml of 2 *N* hydrochloric acid and finally with water until the effluent is chloride-free. Repeat the cycle of alkali, acid and water washing until the final effluent is free from material absorbing at 260 mμ. Do not allow the column to run dry.
Anthrone reagent solution—Dissolve 1 g of anthrone in 1 litre of 76 per cent. v/v sulphuric acid.
Orcinol reagent solution—Dissolve 1 g of orcinol in 100 ml of concentrated hydrochloric acid containing 0·1 g of ferric chloride.

PROCEDURE—

Extraction—Dissect approximately 20 g of fish muscle, and homogenise into 2 volumes of chilled 0·6 *N* perchloric acid. Filter the mixture rapidly at 0° C, and adjust the pH of the filtrate to 7·4 by carefully adding 5 *N* potassium hydroxide. After 30 minutes at 0° C, remove by filtration any potassium perchlorate that has crystallised.

Separation of sugar phosphates—Run the extract through the column of Dowex 1 (chloride), and wash the column with water until the optical density of the effluent is less than 0·01 at 260 mμ. Attach the column to the assembly, and, with the outlet from the mixing vessel closed by means of a screw-clamp, pour 1 litre of the mixing-vessel solution into the mixing vessel. Attach the reservoir to the assembly, and fill it with the reservoir solution. Start the stirrer, and open the reservoir stopcock, the outlet from the mixing vessel and the column stopcock. Collect 140 10-ml fractions at the rate of 6 to 10 fractions per hour, depending on the time available, and measure their optical densities at 260 mμ to establish the positions of the nucleotide peaks. Withdraw 2-ml aliquots from each fraction for the detection and determination of hexose and pentose phosphates by the anthrone[11] and orcinol[14] procedures, respectively.

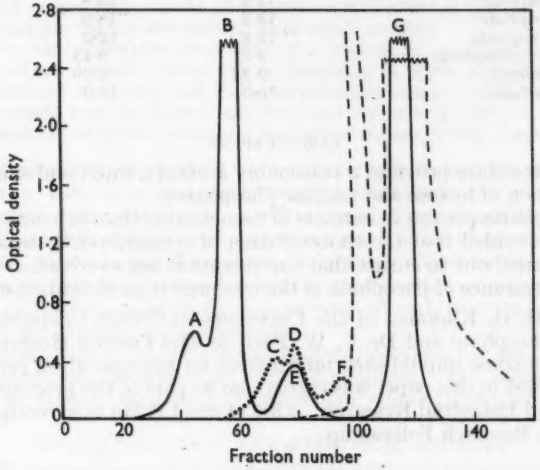

Fig. 3. Chromatographic separation of sugar phosphates present in perchloric acid extract of 20 g of cod muscle: A, glucose 1-phosphate; B, glucose 6-phosphate; C, ribose 1-phosphate; D, unidentified pentose phosphate; E, fructose 1-phosphate; F, ribose 5-phosphate; G, fructose 1:6-diphosphate. Conditions as for Figs. 1 and 2; aliquots treated with anthrone reagent solution (full-line curve); aliquots treated with orcinol reagent solution (dotted-line curve). Optical density of effluent at 260 mμ shown by broken-line curve

RESULTS

The curves in Fig. 3 show the separation of the sugar phosphates present in a perchloric acid extract of cod muscle. The sharp appearance of material absorbing strongly at 260 mμ is a good indication that the elution of virtually all the monophosphates has ended. The three pentose phosphate peaks have not so far been identified from their positions; peaks C and F are believed to be ribose 1-phosphate and ribose 5-phosphate, respectively.

The recoveries of certain sugar phosphates after separation by the proposed procedure are shown in Table I. Commercial samples were used and were obtained from George T. Gurr Ltd., 136 to 138 New Kings Road, London, S.W.6 ("Sigma"—disodium glucose 6-phosphate) and from Courtin & Warner Ltd., Ballards Old Brewery, Lewes, Sussex ("Boehringer"—disodium glucose 1-phosphate monohydrate, barium fructose 1-phosphate, barium fructose 6-phosphate, barium fructose 1:6-diphosphate and barium ribose 5-phosphate dihydrate).

The positions of the peaks obtained were found to vary slightly from one separation to another, but the resolutions were not affected. This shift may be ascribed to different concentrations of anions in the extracts and to ageing of the resin.

Table I

Recoveries of sugar phosphates

Ribose 1-phosphate, as its di*cyclo*hexylammonium salt, was run on to a column (53 cm × 1·8 cm) of Dowex 1–8x (chloride), 200 to 400 mesh. Other compounds were purified by fractionation of the barium salts with ethanol[14]; barium was exchanged for sodium, and the preparations were run on to the column at pH 7·4. Phosphates were eluted by a gradient of 0·19 *M* chloride containing 0·017 *N* hydrochloric acid into 1 litre of 0·01 *M* sodium tetraborate containing 0·0484 *M* chloride. The sugar moieties were determined by reaction with anthrone or orcinol

Compound	Amount applied to column, μmole	Amount recovered, μmole	Recovery, %
Glucose 1-phosphate	13·3	13·8	104
Glucose 6-phosphate	18·2	18·9	104
Fructose 1-phosphate	12·8	12·3	96
Fructose 1 : 6-diphosphate	9·72	9·43	97
Ribose 1-phosphate	9·32	8·29	89
Ribose 5-phosphate	16·0	16·0	100

Conclusions

The proposed procedure provides a reasonably accurate, rapid and simple method for the quantitative separation of hexose and pentose phosphates.

The sugar phosphates present in extracts of tissues other than fish muscle can be separated by this procedure, provided that the concentration of contaminating anions is not too high. Checks should be carried out to ensure that the column is not overloaded when the extract is first applied; the appearance of phosphate in the washings is an indication of such overloading.

We thank Dr. H. G. Khorana, of the University of British Columbia, for the gift of a sample of ribose 1-phosphate and Dr. C. W. Parr, of The London Hospital Medical College, for supplying us with some unpublished information on his separation procedure.

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The Analysis of Bismuth Telluride and Related Thermoelectric Materials

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Methods are presented for analysing thermoelectric materials based on bismuth telluride. The materials examined included modifications of this compound in which bismuth was partly replaced by antimony and tellurium partly replaced by selenium; some materials contained a small amount of iodine.

Bismuth is determined in the presence of the other constituents by direct titration with ethylenediaminetetra-acetic acid.

In the absence of antimony, tellurium is determined volumetrically in the presence of the other constituents by dichromate oxidation of tellurium^{IV} to tellurium^{VI}. When antimony is present a preliminary separation of the tellurium, by precipitation in the elemental form, is effected before completing the determination by the volumetric dichromate method.

For the determination of iodine, the iodine is separated by steam-distillation effected while the sample is dissolving in nitric acid; the distilled iodine is oxidised to iodate and the determination completed by addition of potassium iodide and titration of the liberated iodine with thiosulphate.

The determinations of antimony and selenium are briefly described.

With junctions of dissimilar metals, the thermoelectric effects first observed by Seebeck (in 1821) and by Peltier (in 1834) are too small to be of practical value for the conversion of thermal energy to electrical energy or for the electrical production of heating or cooling effects. However, in recent years it has been found that many materials that are semi-conductors have "Seebeck coefficients" much larger than those of metals. Work in these Laboratories[1] has shown that a few such materials have a favourable combination of properties (high Seebeck coefficient and high ratio of electrical to thermal resistance) such that the thermoelectric effect is of potential value, for example, in refrigeration.

The materials developed in these Laboratories for thermoelectric applications are bismuth telluride, Bi_2Te_3, and various modifications of this compound. The object of this paper is to report the methods devised for analysing thermoelectric materials of this type.

Requirements of the Analytical Methods

The materials it was required to analyse included bismuth telluride and modifications of this compound in which bismuth was partly replaced by antimony and tellurium partly replaced by selenium. Some materials also contained small amounts of iodine, which can have the effect of changing the sign of the thermoelectric properties of the material.

Analyses were required to ascertain the composition of the various materials at different stages of preparation and also to assess the uniformity of composition within a preparation. For these purposes determinations of bismuth, tellurium and, when appropriate, iodine usually sufficed, but determinations of antimony and selenium have also been made.

The development of analytical methods for this work was influenced by three factors: (*i*) the large number of analyses required; (*ii*) a required accuracy of about 1 part in 500 for the major constituents; (*iii*) the need to analyse small specimens. For these reasons rapid volumetric methods involving the minimum of manipulation were sought, capable of giving the required degree of accuracy for the major constituents on sample weights of 0·1 to 0·2 g.

The methods devised for the determination of bismuth, tellurium, iodine, antimony and selenium are discussed below.

Determination of Bismuth

For the direct determination of bismuth in bismuth telluride and related materials the obvious choice was titration with EDTA (ethylenediaminetetra-acetic acid). Bismuth forms one of the most stable of the metal - EDTA complexes and can be titrated with EDTA at acidities as high as 0·1 to 0·2 *N* in nitric acid, thereby avoiding interference from most other metals; xylenol orange is a suitable indicator.[2]

If only bismuth is present, for example, in standardising the EDTA against pure bismuth, the bismuth can be dissolved in a small volume of nitric acid and the titration applied after diluting the bismuth solution to give an acid concentration of about 0·1 *N*. However, with bismuth telluride it was found that dilution after dissolving the sample always resulted in extensive precipitation; the bismuth in the precipitate reacted only slowly with the EDTA titrant, which led to a titration of intolerable duration.

It was found that this difficulty could be overcome by adding EDTA solution to within about 2 ml of the expected titre before dilution with water. Even with this precaution, slight precipitation occasionally occurred and in analysing bismuth telluride it was found advisable to continue mechanical stirring of the solution for a few minutes to ascertain whether the end-point attained was permanent or not.

In determining bismuth in materials containing antimony, slight recurrence of the end-point was always observed, presumably owing to traces of bismuth adsorbed on the antimony precipitate formed by nitric acid attack on the sample. Here again it was advisable to continue stirring for a few minutes to ascertain whether the end-point reached was permanent or not. Addition of tartaric acid to retain the antimony in solution gave less sharp end-points, presumably because of some complexing of the bismuth by the tartaric acid, and titration in the presence of the antimony precipitate was preferred.

TABLE I

RESULTS FOR THE DETERMINATION OF BISMUTH IN THERMOELECTRIC MATERIALS

Sample	Theoretical bismuth content, %	Bismuth found, %
Single-crystal Bi_2Te_3	52·2	52·1, 52·2, 52·2
Known Bi - Sb - Te - Se mixture	44·6	44·5, 44·6, 44·7
Known Bi - Sb - Te mixture	15·1	15·1

With 0·01 *M* EDTA (1 ml ≡ about 2 mg of bismuth) as titrant, the required accuracy could be obtained with sample weights of about 0·1 g. Results obtained on single crystals of pure bismuth telluride and on known mixtures containing antimony, tellurium and selenium are shown in Table I.

Details of the procedure for bismuth are given below.

METHOD FOR DETERMINING BISMUTH—

Special reagents—

EDTA solution, approximately 0·01 M—Dissolve 7·445 g of disodium ethylenediaminetetra-acetate dihydrate in water, and dilute to 2000 ml. Standardise by applying the procedure described below to accurately weighed amounts (about 0·1 g) of pure bismuth.

Xylenol orange indicator solution—Dissolve 0·1 g of xylenol orange in 50 ml of water. The solution slowly deteriorates and should be renewed monthly.[3]

Procedure—

Weigh accurately 0·10 to 0·11 g of the sample, previously ground to a fine powder in an agate mortar. Transfer the weighed sample to a 200-ml beaker, cover the beaker, and add 1·5 ml of diluted nitric acid (1 + 1).

Stand the beaker at an angle so that it touches a hot-plate at only one point, and allow the beaker to remain in this position until no trace of unattacked sample can be seen. Heating in this manner minimises the evaporation of acid during decomposition of the sample, which may require 10 to 15 minutes for samples rich in antimony.

When the attack appears complete, swirl the solution to dissolve any soluble salts that may have crystallised at the edge of the solution. Add about 0·5 ml of water, and, with the beaker flat on the hot-plate, heat the solution for 1 minute to decompose any undetected residual traces of sample that may be obscured by the antimony precipitate, if present. Finally, add 3 ml of water, boil the solution for 1 minute, and then cool.

Without any further addition of water, run in the EDTA solution from a burette until the volume added is about 2 ml less than the expected titre; swirl the solution during the addition. Rinse the cover with water, add the rinsings to the solution, and then add sufficient water to give a volume of 100 to 120 ml.

Add 4 drops of xylenol orange indicator solution, transfer the beaker to a magnetic stirrer, and titrate the stirred solution slowly with EDTA until the colour of the indicator changes completely from red to yellow. Continue stirring for 1 to 2 minutes, and, if necessary, add a further drop of titrant to restore the yellow colour of the indicator. Continue stirring for a further 5 minutes, add 1 drop of titrant, and include this in the titre if any change of colour is observed.

From the total titre, calculate the bismuth content of the sample.

NOTE—In the presence of much antimony, appreciable adsorption of the indicator by the antimony precipitate can occur at the first apparent end-point. For this reason, if much antimony is present, it is preferable to add only 2 drops of indicator initially and to add a further 2 drops when the first apparent end-point is reached.

DETERMINATION OF TELLURIUM

The dichromate method[4] appears to be the only volumetric procedure for tellurium not subject to interference from selenium. The method involves oxidation of tellurium^{IV} to tellurium^{VI}, usually in a hydrochloric acid medium, by addition of excess of dichromate solution; after a standing period to achieve complete oxidation, ammonium ferrous sulphate solution is added in an amount greater than that required to reduce the excess of dichromate, and the determination is completed by titration with dichromate of the residual ferrous salt.

For trials of this method on thermoelectric materials, the sample was first dissolved in nitric acid, thereby obtaining the tellurium in the quadrivalent state; after expulsion of nitrates by evaporation with hydrochloric acid, the dichromate method was applied in hydrochloric acid medium as recommended by the original workers.[4] This process behaved satisfactorily with bismuth telluride, but gave fallacious results when antimony was present. This effect appeared to arise from partial reduction of quinquivalent antimony on addition of the ferrous solution, the tervalent antimony so formed not being immediately re-oxidised in the subsequent final titration with dichromate.

Consideration of other redox systems suggested that this difficulty was unlikely to be overcome other than by removal of the antimony or prior isolation of the tellurium. Precipitation of tellurium as the element was the obvious method of effecting the required separation.

The procedure described below was then adopted for determining tellurium in the presence of antimony. The sample was decomposed with nitric acid and the solution evaporated and re-evaporated with hydrochloric acid; elimination of nitrates is essential for complete recovery of tellurium in the subsequent precipitation. The tellurium was then precipitated with hydrazine hydrochloride and sulphurous acid,[5] the filtered, washed precipitate was re-dissolved in nitric acid and the tellurium determined by the volumetric dichromate method. The volumetric procedure was applied in a nitric - sulphuric acid medium, as used in the A.S.T.M. method for tellurium in copper alloys,[6] thereby avoiding the evaporations that would have been necessary to convert to a hydrochloric acid medium. Use of approximately 0·05 *N* dichromate (1 ml ≡ 3 mg of tellurium) permitted sample weights of about 0·2 g to be employed.

A crucial part of this procedure was found to be the filtering and washing of the bulky tellurium precipitate under suction. Erratic results could be obtained if the mat of tellurium in the sintered-glass crucible were allowed to suck dry during the process, so that the mass compacted and cracked. A possible explanation was that the resultant channelling through the precipitate led to inefficient washing and that traces of unremoved adsorbed material, *e.g.*, hydrazine or antimony salts, caused errors in the final volumetric procedure. By operating under gentle suction it was readily possible to filter and wash the tellurium precipitate without its compacting or sucking dry, and the procedure outlined then gave accurate and precise results for tellurium.

The upper part of Table II shows results obtained in this manner on known mixtures containing bismuth, antimony and selenium. These figures show that the method affords the required accuracy. As expected, the presence of minor amounts of selenium, although co-precipitated with the tellurium, did not affect the accuracy of the method. It was, however, observed that the selenium in the precipitate dissolved less readily in the nitric acid than did the tellurium; this might cause some manipulative difficulty for compounds in which selenium predominated.

The lower part of Table II shows the results obtained by direct volumetric determination of tellurium in single-crystal bismuth telluride. In the direct determination, applicable when

TABLE II

RESULTS FOR THE DETERMINATION OF TELLURIUM IN THERMOELECTRIC MATERIALS

Sample	Theoretical tellurium content, %	Tellurium found, %
(*a*) *Volumetric determination after separation of tellurium*—		
Known Bi - Sb - Te mixtures ..	55·7	55·8
	56·1	56·0
	55·1	55·1
	57·0	57·2
Known Bi - Sb - Te - Se mixtures	47·6	47·5, 47·5
	56·9	56·8, 56·9
(*b*) *Direct volumetric determination*—		
Single-crystal Bi_2Te_3	47·8	47·7, 47·8, 47·7

antimony is absent, the ability to effect the volumetric procedure in nitric - sulphuric acid medium is of particular advantage; the sample can be dissolved in nitric acid and the volumetric procedure applied without any need for evaporations or separations.

Details of the methods for tellurium in the presence and absence of antimony are given below.

METHOD FOR DETERMINING TELLURIUM—

Special reagents—

Standard potassium dichromate solution—Dissolve 4·611 g of potassium dichromate, previously dried at 150° C, in water, and dilute to 2000 ml. This solution is used as a primary standard.

1 ml ≡ 3·000 mg of tellurium.

Ammonium ferrous sulphate solution, approximately 0·1 N—Dissolve 78·4 g of hydrated ammonium ferrous sulphate in a mixture of 900 ml of water and 100 ml of sulphuric acid, sp.gr. 1·84; cool, and dilute with water to 2000 ml.

Barium diphenylamine sulphonate solution, 0·3 per cent. w/v, aqueous.

Sulphur dioxide solution, saturated—Prepare freshly before use.

Hydrazine hydrochloride solution, 15 per cent. w/v, aqueous—Prepare freshly before use.

Procedure—

(*i*) *Antimony absent*—Use an amount of the ground sample expected to contain 100 to 120 mg of tellurium. Transfer the weighed sample to a 400-ml beaker, add 10 ml of diluted nitric acid (1 + 1), and warm to dissolve the sample. When solution is complete, add about 40 ml of water, boil for 1 minute, and then cool.

Add 1 g of urea to eliminate any nitrous acid, stir to dissolve the solid, and then add 140 ml of dilute sulphuric acid (1 + 6). Add from a pipette 50 ml of the standard potassium dichromate solution, and set the mixture aside for 30 to 45 minutes to ensure complete oxidation of the tellurium.

When this period has elapsed, add from a pipette 10 ml of ammonium ferrous sulphate solution; then add 5 ml of phosphoric acid, sp.gr. 1·75, and 10 drops of barium diphenylamine sulphonate solution, and titrate with the potassium dichromate solution to the purple end-point.

On the same day as the tellurium determination, ascertain the dichromate equivalence of the ferrous solution as follows. To 10 ml of diluted nitric acid (1 + 1) add, successively, 40 ml of water, 1 g of urea, 140 ml of dilute sulphuric acid (1 + 6) and 50 ml of water. Add from a pipette 10 ml of ammonium ferrous sulphate solution; then add 5 ml of phosphoric acid and 10 drops of barium diphenylamine sulphonate solution, and titrate with the potassium dichromate solution to the purple end-point.

Subtract the volume of dichromate solution required for this titration from the total volume of dichromate solution added in the determination of tellurium, thus obtaining the volume equivalent to the tellurium present. Hence calculate the tellurium content of the sample.

(*ii*) *Antimony present*—Use an amount of the ground sample expected to contain 100 to 120 mg of tellurium. Transfer the weighed sample to a 250-ml beaker, add 5 ml of diluted

nitric acid (1 + 1), and heat the covered beaker on a hot-plate until no trace of unattacked sample can be seen. Rinse the cover, add the rinsings to the solution, transfer the beaker to a steam-bath, and allow the solution to evaporate to dryness.

Moisten the residue with hydrochloric acid, sp.gr. 1·18, cover the beaker, and warm on the steam-bath to dissolve the residue. Rinse the cover, add the rinsings to the solution, and then add sufficient hydrochloric acid to re-dissolve any hydrolysis product formed during the rinsing. Allow the solution to evaporate on the steam-bath until no smell of hydrochloric acid can be detected.

Add 10 ml of hydrochloric acid, cover the beaker, and warm on the steam-bath to dissolve the pasty residue. Dissolve 1 g of tartaric acid in 40 ml of hot water, add this to the solution, and heat the mixture almost to boiling; the solution should be free from insoluble matter at this stage.

Add 15 ml of freshly prepared saturated sulphur dioxide solution, 10 ml of freshly prepared hydrazine hydrochloride solution and then a further 25 ml of sulphur dioxide solution, stirring while each addition is made. Heat the solution to boiling, and continue boiling for 5 minutes to obtain a flocculent precipitate.

Set the solution aside for about 15 minutes without heating, and then collect the tellurium precipitate on a small No. 4 grade sintered-glass crucible under gentle suction. With hot dilute hydrochloric acid (1 + 49) wash into the crucible as much as possible of the precipitate, and then wash the crucible and precipitate three times. Rinse the cover and beaker well with hot water to remove chlorides, pouring the rinsings into the crucible, and then wash the crucible and precipitate three times with hot water. During the filtration and washing take particular care that the mat of tellurium in the crucible does not compact and suck dry, otherwise inefficient washing, leading to errors in the determination, can occur.

When the washing is complete, increase the suction and suck the precipitate as dry as possible so that the bulk of the precipitate becomes loose in the crucible. Tip as much as possible of the precipitate into the beaker in which the precipitation was made, and add to the contents of the beaker 5 ml of diluted nitric acid (1 + 1). Heat the covered beaker to dissolve the precipitate.

Dissolve the portion of precipitate remaining in the crucible by passing through the crucible 5 ml of hot diluted nitric acid (1 + 1), added in small portions, then wash the crucible several times with hot water. (In the presence of selenium, which dissolves less readily in the diluted acid, add 3 ml of hot diluted nitric acid (1 + 1) to dissolve most of the precipitate in the crucible, and then add dropwise 1 ml of hot concentrated nitric acid to dissolve the remaining precipitate; finally wash with hot water).

Transfer to a 400-ml beaker the two solutions containing dissolved precipitate; the volume should be about 50 ml at this stage. Boil the solution for 1 minute, and then cool. Add 1 g of urea, stir to dissolve the solid, and add 140 ml of dilute sulphuric acid (1 + 6).

Add from a pipette 50 ml of standard potassium dichromate solution, and complete the determination as described for the procedure in the absence of antimony.

Determination of iodine

It was required to determine concentrations of the order of 0·1 per cent. of iodine, present as an additive in bismuth telluride and certain related materials. An accuracy to within 1 or 2 per cent. of the iodine content was sought, preferably attainable with sample weights of less than 1 g.

For the final volumetric determination of the iodine, the procedure used was essentially that devised by Leipert for the micro-determination of iodine in organic materials after combustion.[7,8] The iodine, absorbed in sodium hydroxide solution, is oxidised to iodate with a bromine - acetic acid - sodium acetate reagent, and excess of bromine is eliminated with formic acid; after addition of potassium iodide and sulphuric acid the liberated iodine is titrated with thiosulphate. The advantage of this procedure is the gain in sensitivity resulting from the conversion to iodate; 6 atoms of iodine are liberated for each atom of iodine originally present.

For the necessary preliminary separation of the iodine, steam-distillation was used. This process is normally applied to an acidified iodide solution after addition of nitrite, the free iodine liberated by the action of nitrous acid being carried over in the current of steam and absorbed in sodium hydroxide solution.[9] In the work on thermoelectric materials it was

found unnecessary to add nitrite if the solution of the sample in nitric acid and the steam-distillation were allowed to proceed simultaneously, provided that a suitable concentration of nitric acid was used.

With the process applied in this manner, oxides of nitrogen liberated during solution of the sample were absorbed by the sodium hydroxide solution used to trap the distilling iodine. This was found to lead to an appreciable "bromine demand" when the distillate was oxidised with bromine for the volumetric determination of iodine. Although this oxidation stage would presumably convert to the nitrate form the oxides of nitrogen absorbed in the distillate, no interference with the determination of iodine occurred provided that a suitable concentration of nitric acid was used to dissolve the sample. Too high a concentration of acid at the dissolving stage led to an abnormally high "bromine demand" of the distillate and positive errors in the determination of iodine could then occur, attributable to liberation of iodine from potassium iodide owing to the higher concentration of nitrate present. Addition of nitrite to the dissolving solution could give a similar effect. On the other hand, too low a concentration of nitric acid at the dissolving stage led to low results for iodine, presumably owing to incomplete conversion of the dissolved iodine to the elemental form.

With 0·5 g of sample, a suitable dissolving mixture that avoided these errors was 4 ml of water *plus* 5 ml of nitric acid, sp.gr. 1·42. Alteration of the amount of water to 3 or 6 ml could cause small positive or negative errors, respectively.

Use of the "correct" dissolving mixture permitted determination of iodine with the required accuracy, as shown by the results in Table III. The steam-distillation is simple and rapid to perform; a determination can be completed in about 30 minutes.

Details of the method for iodine are given below.

TABLE III

RESULTS FOR THE DETERMINATION OF IODINE IN THERMOELECTRIC MATERIALS

Sample	Theoretical iodine content, %	Iodine found, %
Known Bi - Sb - Te - Se - I mixtures	0·063	0·064, 0·062
	0·127	0·126, 0·125, 0·127
	0·190	0·190, 0·193

METHOD FOR DETERMINING IODINE—

Distillation apparatus—

A round-bottomed 50-ml flask with a B24 neck is fitted with a "recovery head" having an upper B14 socket and a B19 cone on the side-arm. Into the upper socket of this is fitted a B14 delivery tube to which an extra length of tube has been sealed so that the delivery tube almost touches the bottom of the flask. The upper end of the delivery tube is connected by rubber tubing to a steam generator. The side-arm of the recovery head is connected to a B19 receiver adapter (long plain-bend type) that dips to the bottom of a boiling tube used to collect the distillate. The boiling tube is immersed to an appropriate depth in a beaker of water to cool the distillate.

Special reagents—

Sodium hydroxide solution, 5 per cent. w/v—Store in a polythene bottle.

Liquid bromine—Analytical-reagent grade of negligible iodine content.

Sodium acetate in acetic acid—Dissolve 40 g of sodium acetate trihydrate in 400 ml of glacial acetic acid.

Sodium acetate solution—Dissolve 80 g of sodium acetate trihydrate in 400 ml of water.

Formic acid, 90 per cent. w/w—Store in a dropping bottle.

Starch indicator solution—Dissolve 1 g of sodium starch glycollate in 100 ml of water with warming.

Standard sodium thiosulphate solution, approximately 0·01 N—Prepare by dilution of standardised 0·1 *N* sodium thiosulphate. If required, standardise the diluted thiosulphate solution against 0·01 *N* potassium dichromate. One millilitre of 0·01 *N* thiosulphate is equivalent to 0·2115 mg of iodine being determined.

Procedure—

Transfer 0·5 g of the ground sample to the dry 50-ml flask of the distillation apparatus, and add 4 ml of water. Assemble the distillation apparatus, but do not connect the supply

of steam; ensure that the water in the steam generator is boiling. Add 5 ml of sodium hydroxide solution and 10 ml of water to the boiling tube, which serves as receiver.

Carefully add 5 ml of nitric acid, sp.gr. 1·42, down the delivery tube into the flask, and immediately connect the supply of steam. Pass in steam until the total volume in the receiver reaches about 30 ml, and then disconnect the supply of steam. The rate of passage of steam should be such that the distillation takes about 10 minutes. Transfer the solution in the receiver to a 150-ml flask fitted with a ground neck, and add the washings from the receiver adapter. Cool the flask and contents.

Dissolve 10 drops of bromine in 4 ml of sodium acetate in acetic acid, add the mixture to the solution in the 150-ml flask, and then add 5 ml of sodium acetate solution. Mix, and set the solution aside for 1 minute.

Add 3 drops of formic acid down the neck of the flask, and swirl for 30 seconds. If the yellow colour of free bromine has not been completely discharged, add 1 further drop of formic acid, and swirl for 15 seconds. Add a small drop of methyl red indicator solution by means of a drawn-out glass tube. If the indicator colour is discharged by free bromine present, add a further drop of formic acid, swirl for 15 seconds, and again test by adding a small drop of methyl red indicator. A total addition of 4 to 6 drops of formic acid is usually sufficient to eliminate the excess of bromine.

Add 0·2 g of potassium iodide and then 2 ml of dilute sulphuric acid (1 + 6), and immediately insert the stopper. Swirl to dissolve the iodide, and set the solution aside for 5 minutes.

When this time has elapsed, rinse the stopper with 2 to 3 ml of water, collecting the rinsings in the flask. Rapidly titrate with the standard sodium thiosulphate solution until the iodine colour is almost discharged. Add 1 ml of the starch indicator, and continue the titration dropwise until the blue colour is just discharged. Because of the presence of methyl red the final colour change is from blue to pink.

From the titre calculate the iodine content of the sample.

NOTES—1. Although the recovery head serves as a splash trap, care should be taken to avoid carry-over of sample solution during the distillation. The presence of antimony, for example, in the distillate will interfere with the iodine determination.

2. The amount of bromine specified should be sufficient to oxidise all reducing species in the distillate and to provide about 2 drops in excess.

DETERMINATION OF ANTIMONY AND SELENIUM

As stated earlier, determination of bismuth, tellurium and, when appropriate, iodine, usually sufficed, but some determinations of antimony and of selenium in thermoelectric materials have also been made.

When required, the determination of antimony was effected in conjunction with that of tellurium. The filtrate from the tellurium precipitation was treated with hydrogen sulphide to precipitate the antimony. The antimony precipitate, which also contained any bismuth present, was separated by filtration and dissolved by heating with a mixture of sulphuric acid and potassium sulphate. After treatment with sulphur dioxide to ensure that the antimony was wholly in the tervalent state, the determination was completed by permanganate titration of the antimony in cold sulphuric - hydrochloric acid medium.[10] Results for major amounts of antimony agreed well with the values calculated by difference after determination of the other constituents.

For the determination of minor amounts of selenium, the sample was dissolved in nitric acid, the solution evaporated to dryness and the residue re-dissolved in hydrochloric acid (3 + 1). Selenium was precipitated as the element by passage of sulphur dioxide through the cold solution, and the precipitate was filtered, dried and weighed. Operation at this high concentration of acid permits the selective precipitation of selenium in the presence of tellurium.[11]

DISCUSSION OF THE METHODS

During the preparation of this paper for publication, a relevant paper by Reed[12] was published, describing a somewhat different approach to the analysis of similar thermoelectric materials. Apart from antimony, determined in the filtrate from the tellurium precipitation, our procedures for each constituent are independent, whereas Reed's method comprises a scheme for the consecutive determination of selenium, tellurium, antimony and bismuth

on the same portion of sample. In this scheme selenium is first determined gravimetrically by precipitation with sulphurous acid from hydrochloric acid (4 + 1) solution. In the filtrate tellurium is determined gravimetrically by precipitation with hydrazine hydrochloride and sulphurous acid from 3 *M* hydrochloric acid. The filtrate from the tellurium determination is divided into two portions, one being used for determination of antimony by sulphide precipitation, solution of the precipitate and final potentiometric titration of the antimony with permanganate. In the other portion of the filtrate bismuth is determined by titration with EDTA, thiourea being used as indicator.

Our procedures therefore resemble those of Reed for selenium and antimony, but differ somewhat for bismuth and tellurium. Reed states that his procedure for bismuth cannot be directly applied because tellurium reacts with the indicator, thiourea. This difficulty does not arise in our method in which the indicator is xylenol orange.

In Reed's method for tellurium, the filtered precipitate is dried *in vacuo* at room temperature to avoid oxidation of the tellurium, which is stated by Duval[13] to occur at temperatures above 40° C. In this context Duval's observation is not wholly relevant, his thermogravimetric experiments being made on the "finely divided" precipitate obtained by precipitation with hydrazine alone; precipitation with hydrazine and sulphurous acid, as used by Reed and ourselves, yields a much more flocculent precipitate. In experiments on pure tellurium solutions we found that the weights of tellurium precipitates, dried for 1 hour at 105° C, exceeded the theoretical weights by about 6 parts per 1000. These positive errors were presumably due to slight oxidation of the tellurium at some stage in the procedure, although textbook descriptions of the method usually refer to the washing, but not the drying, as the stage at which the tellurium is particularly susceptible to oxidation. Further work showed that solution of these "oxidised" precipitates, followed by volumetric determination, gave correct values for tellurium. The volumetric finish thus provides a means of avoiding the gravimetric errors that can undoubtedly arise from oxidation of the tellurium precipitate, whether this occurs in washing or drying.

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The Micro-determination of Ferrous Iron in Silicate Minerals by a Volumetric and a Colorimetric Method

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Methods are described for determining ferrous iron in milligram amounts of sample. Decomposition is effected at room temperature by hydrofluoric acid containing quinquivalent vanadium to oxidise ferrous iron as it passes into solution. In the volumetric method, the ferrous iron is determined by titrating the excess of vanadium^{V}. The colorimetric determination is carried out by utilising the reversibility of the reaction $V^{5+} + Fe^{2+} \rightleftharpoons V^{4+} + Fe^{3+}$, and the ferrous iron re-formed is determined by means of dipyridyl.

In the determination of the ferrous iron contents of silicate rocks and minerals, measures have to be taken to prevent atmospheric oxidation during decomposition. For example, a sealed tube is used in the decomposition of resistant silicates by sodium fluoroborate flux. When the sample is decomposed by hot or boiling hydrofluoric and sulphuric acids,[1] an atmosphere of steam or steam *plus* carbon dioxide is used to prevent atmospheric oxidation of ferrous iron, which is pronounced in the presence of fluoride ions, presumably owing to the formation of ferric fluoride complexes.

An alternative approach, in which an inert atmosphere is not necessary, is the method developed by Wilson for silicate rocks[2]; quinquivalent vanadium is used to oxidise the ferrous iron as it passes into solution. The stability of the oxides of vanadium in boiling sulphuric acid has been discussed by Sieverts and Muller,[3] and Schein[4] developed a method for determining ferrous iron in chromites. Schein's method involved use of hot sulphuric and orthophosphoric acids and vanadium pentoxide, which is moderately stable under these conditions. Wilson found that quinquivalent vanadium was stable in hydrofluoric acid for up to 3 weeks at room temperature and so used hydrofluoric acid under these conditions to open up silicate rocks[2]; Jackson has adapted this method for a similar determination on slags.[5]

Of these methods, that described by Rowledge[1] has been adapted for the micro-determination of ferrous iron in resistant minerals by Hey.[6] Of the methods involving decomposition by hydrofluoric and sulphuric acids, the most satisfactory in general use, that developed by Pratt,[1] requires a 100-ml platinum crucible for good results, as air is excluded by vigorously boiling the acids to provide an atmosphere of steam. As this procedure, by the nature of its technique, is not suitable for adaptation to the micro scale, the alternative method, in which vanadium^{V} is used, has been adapted to the micro scale for volumetric and colorimetric determinations.

Development of volumetric method

In the original macro method for silicate rocks,[2] 0·5000 g of sample was decomposed at room temperature by 10 ml of hydrofluoric acid containing vanadium^{V}, the excess of which was then determined by titration with ammonium ferrous sulphate, barium diphenylamine sulphonate being used as indicator. Boric acid was used to form a complex with the fluoride ions and served the double purpose of bringing insoluble fluorides formed during the decomposition back into solution and of protecting glassware against attack. Boric acid has limited solubility, and comparatively large volumes had to be used; the final volume of solution was about 250 ml.

A simple scaling down of this method was not sufficient, as the end-point with barium diphenylamine sulphonate became too diffuse in the less concentrated solutions; the addition of orthophosphoric acid also failed to make the end-point sharp. However, it was found that, if the titration was carried out in the presence of free fluoride ions, the end-point was considerably improved and became sharp, as little as 0·0001 ml of 0·139 *N* solution being sufficient to produce a definite change in colour. This is probably because of the formation of ferric fluoride complexes, which decreases the Fe^{3+} - Fe^{2+} oxidation potential by removing ferric ions from solution. When polystyrene vessels were used throughout, it was possible to dispense with the addition of boric acid, and it was found that insoluble fluorides could be

dissolved and kept in solution by maintaining the acidity of the solution, at any stage, not less than 4 *N* in sulphuric acid. At lower concentrations of acid, fluorides tend to be thrown out of solution and may interfere with the titration. The final titration was made with a comparatively concentrated solution of ammonium ferrous sulphate (0·139 *N*, *i.e.*, 1 ml equivalent to 10 mg of FeO) delivered from a micrometer-syringe burette. The final volume of solution was kept down to 7 ml, thereby making possible the use of the same polystyrene vessel for both decomposition and titration and so avoiding transference of the solution.

STABILITY OF QUINQUIVALENT AND QUADRIVALENT VANADIUM—

The method depends on vanadium^{V} not being reduced by hydrofluoric acid and on vanadium^{IV} not being oxidised by air. The stabilities of these ions were tested by placing 0·1000-ml portions of 0·139 *N* solutions in polystyrene vessels containing 1 ml of hydrofluoric acid; the results in Table I show the titres found after different times of standing and demonstrate the stabilities of the ions.

TABLE I

STABILITIES OF VANADIUM IONS IN HYDROFLUORIC ACID

Time of standing in hydrofluoric acid, hours	Titre of 0·139 *N* ammonium ferrous sulphate for solution containing 0·1000 ml of—	
	0·139 *N* V^{4+}, ml	0·139 *N* V^{5+}, ml
0	0·0000	0·1000
4		0·1000, 0·1000, 0·1000
24		0·1000, 0·1000, 0·1000
72		0·1000, 0·0996, 0·1000

For periods longer than 72 hours, recoveries of vanadium^{V} were erratic, possibly owing to "creeping" of the solution. Such long periods of standing, however, are not generally required, since most rocks and minerals are decomposed within 4 hours; comparatively few samples need be set aside overnight.

The recovery of ferrous iron was tested by adding various amounts of 0·139 *N* ammonium ferrous sulphate to solutions consisting of 0·1000-ml portions of 0·139 *N* quinquivalent vanadium and 1 ml of hydrofluoric acid and setting the mixtures aside overnight before titration; the results are shown in Table II.

TABLE II

RECOVERY OF FERROUS IRON

Volume of ammonium ferrous sulphate solution initially added, ml	Titre of ammonium ferrous sulphate solution, ml	Total volume of ammonium ferrous sulphate solution added,* ml
0·0000	0·1000	0·1000
0·0100	0·0898	0·0998
0·0300	0·0702	0·1002
0·0900	0·0102	0·1002

* Theoretical volume needed, 0·1000 ml.

METHOD

APPARATUS—

Polystyrene weighing bottles—Capacity 20 ml; 50 mm high × 20 mm diameter.

Agla micrometer-syringe burette.

Magnetic stirrer—The stirring rods are lengths of iron wire sealed in polythene tubes about 15 mm long.

REAGENTS—

Quinquivalent vanadium solution, 0·139 N—Prepare by dissolving ammonium vanadate in sulphuric acid, the final solution being 2 *N* in this acid. Use this solution as the working standard. The solid ammonium vanadate used was of analytical-reagent grade and was standardised against potassium dichromate via a solution of ammonium ferrous sulphate; it was found to have a factor of 0·996, which remained constant over 5 years. Solutions used were stable for at least 1 year.

Ammonium ferrous sulphate, 0·139 N, *in* N *sulphuric acid*—1 ml of this solution is equivalent to 10·0 mg of FeO.

Barium diphenylamine sulphonate indicator solution, 0·01 *per cent. w/v, aqueous.*

Hydrofluoric acid, 40 *per cent. w/v.*

Sulphuric acid, 10 N.

PROCEDURE—

Accurately weigh 3 to 20 mg of ground mineral sample (—70 mesh), and transfer to a polystyrene vessel. Add 0·1000 ml of 0·139 *N* vanadium^{V} solution from a micrometer-syringe burette; amounts of ferrous iron greater than 1 mg, as FeO, require more vanadium^{V} solution. Add 1 ml of hydrofluoric acid, and set aside; most minerals will be decomposed within 4 hours, a few will require digestion overnight, but, for more resistant minerals, up to 3 days' standing is permissible.

When decomposition is complete, add 3 ml of 10 *N* sulphuric acid, and stir the solution with a magnetic stirrer to dissolve any insoluble fluorides that may have been precipitated. Add 5 drops of barium diphenylamine sulphonate indicator solution and 3 ml of water, and titrate with 0·139 *N* ammonium ferrous sulphate added from a micrometer-syringe burette, with magnetic stirring. The end-point is a sharp change from deep purple to pale green. Carry out a control experiment on the reagents, and calculate the ferrous iron content of the mineral from the difference between the two titres.

DEVELOPMENT OF COLORIMETRIC METHOD

In developing a colorimetric method, use was made of the reversibility of the reaction—

$$V^{5+} + Fe^{2+} \rightleftharpoons V^{4+} + Fe^{3+}.$$

The direction of this reaction is dependent on pH. In strongly acid solution it proceeds quantitatively from left to right, but, if the pH is increased sufficiently, ferric ions are reduced to the bivalent state by quadrivalent vanadium. It is therefore possible to develop a direct colorimetric determination by using vanadium^{V} to oxidise ferrous iron during decomposition by hydrofluoric acid and then increasing the pH to regenerate the ferrous iron, which can then be determined colorimetrically by any of the usual reagents. These reagents have the advantage that they may be used in weakly acid solutions, *e.g.*, at pH 5, provided by an acetate buffer, in which the reduction of iron^{III} by vandium^{IV} takes place readily; the formation of a coloured ferrous complex also helps this reaction. It is necessary to convert the free fluoride ions into a complex, as these would tend to hold the iron in the tervalent state by complex formation. Beryllium sulphate was preferred to boric acid for this purpose, as it is much more soluble in water and appears to be more effective in bringing insoluble fluorides into solution. 2:2′-Dipyridyl was used as the colorimetric reagent for ferrous iron.

RECOVERY OF FERROUS IRON

To establish whether or not recovery was quantitative, various amounts of ferrous iron, as ammonium ferrous sulphate, were treated by the colorimetric procedure outlined above and the optical densities found were compared with those obtained by the conventional colorimetric procedure when the hydrofluoric acid, vanadium^{V} and beryllium sulphate were omitted. Measurements were made at 525 mμ in 1-cm cells and corrected for blank values; the results, which indicated that recovery was complete, were—

Iron added per 100 ml of solution, as FeO, μg	400	320	240	160	80
Optical density by conventional procedure ..	0·487	0·388	0·291	0·195	0·097
Optical density by proposed procedure ..	0·487	0·389	0·291	0·191	0·095

METHOD

REAGENTS—

As for the volumetric method, together with—

2:2′-Dipyridyl solution, 0·15 *per cent. w/v, aqueous.*

Beryllium sulphate solution, 50 *per cent. w/v, aqueous*—Prepare from beryllium sulphate tetrahydrate.

Ammonium acetate solution, 50 *per cent. w/v.*

PROCEDURE—

Carry out the decomposition as in the volumetric method, except that, as the vanadium^{V} solution need not be measured with high accuracy, a capillary pipette can be used to add 0·10

ml of this solution. When decomposition is complete, add 5 ml of beryllium sulphate solution, and stir until all insoluble curdy precipitates have dissolved. Wash the contents of the polystyrene vessel into a 100-ml calibrated flask containing 5 ml of 2:2′-dipyridyl solution and 10 ml of ammonium acetate solution diluted to about 50 ml with water (this ensures a final pH of 5). Dilute to 100·0 ml, and, after 10 minutes, measure the optical density of the solution at 525 mμ in a 1-cm cell with a Hilger Uvispek spectrophotometer; use distilled water in a similar cell as reference. Deduct the reagent blank (about 0·040) from this value, and carry out a control determination with a sample of silicate rock having a known ferrous iron content as a check on the calibration.

Results

The proposed methods were applied to samples from the Geological Survey collection of analysed rocks, the ferrous iron contents of which had been determined by other workers on the macro scale by a modified version of Pratt's method. The standard rocks granite G-1 and diabase W-1 were also examined; the results are shown in Table III.

Table III

Results for ferrous iron by various methods

Serial No.	Rock material	Ferrous oxide content found by— proposed volumetric method, %	proposed colorimetric method, %	modified Pratt method, %	Pratt's method in other laboratories, %	Approximate minimum time for decomposition, hours
982	Anthophyllite-cordierite-granulite, St. Keverne, Cornwall	6·38	6·35, 6·39	6·37	—	2
1626	Lamprophyre, Loch Sunart, Argyllshire	4·40	4·35, 4·38	4·40	—	
1732	Biotite-granite, Morvern, Argyllshire	0·93	0·95, 0·92	0·92	—	4
1771	Granite, Burn of Roerwater, Shetland	0·33	0·33, 0·33	0·33	—	
G-1	Granite, Westerly, Rhode Island, U.S.A.	0·93	0·91, 0·94	0·93	0·93,* 0·95†	
W-1	Dolerite (diabase), Centerville, Fairfax County, Virginia, U.S.A.	8·77	8·73, 8·75	8·67	8·65,* 8·7†	2
1518	Sideritic mudstone, Whissendine, Leicestershire	41·9	41·5, 41·8	41·7	—	<½

* Results found by Riley.[7]
† Results found by Shapiro and Brannock.[8]

The colorimetric method was applied to further rocks having high ratios of ferric to ferrous iron; this ratio had no effect on the determination unless it exceeded 20 to 1. Under these conditions, and when comparatively large amounts of ferric iron were present, a faint

Table IV

Ferrous iron found in samples having high ratio of ferric to ferrous iron

Serial No.	Rock material	Ferric iron content, as Fe_2O_3, %	Ferrous iron content, as FeO, %	Ferrous iron found by proposed colorimetric method, as FeO, %	Approximate minimum time for decomposition, hours
1527	Olivine-basalt, Arngask, Perthshire	8·29	0·33	0·32, 0·27	6
1696	Rhyolite, Church Stretton, Shropshire	3·58	0·71	0·70, 0·68	2
1711	Aegirine-granite, Rockall, North Atlantic	8·32	2·44	2·46, 2·52	30
979	Olivine-basalt, Stonehaven, Kincardine-shire	9·31	0·72	0·73, 0·69	1
1579	Olivine-basalt, Skye, Inverness-shire	18·55	0·82	0·82, 0·80	50
1708	Haematite-ironstone	74·32	1·05	1·00, 1·00	2

yellow colour developed, possibly due to ferric acetate, which made results slightly high. For such samples, a small correction was applied by measuring the yellow colour at 420 mμ, at which wavelength it was more intense and the colour of the ferrous - dipyridyl complex was much reduced. The results in Table IV show that the method is reliable for ratios of ferric to ferrous iron up to 75 to 1; when the ratio exceeded 20 to 1, a correction was applied as mentioned above.

Interference

As in all methods for determining ferrous iron, oxidising and reducing substances, such as organic carbon, pyrolusite, sulphides of iron and oxides of vanadium, would be expected to interfere, although these contaminants are seldom encountered in minerals, as distinguished from rocks.[1] The interference from a sample of pyrite and a sample of pyrrhotite was therefore examined and compared with the interference found when the Pratt method was used. By the colorimetric method, the apparent ferrous oxide content of the pyrite was found to be between 5 and 8 per cent. (depending on the time of standing, which was up to 24 hours); by the Pratt method, the apparent ferrous oxide content was 4 to 5 per cent. The apparent ferrous oxide content of the pyrrhotite varied from 1·5 to 4 per cent. (again depending on the time of standing, which was up to 24 hours), compared with 53 to 54 per cent. found by the Pratt method.

The vanadium content of many minerals and rocks is so low, *e.g.*, in granitic rocks it is often much less than 50 to 100 p.p.m.,[9] that its effect on the determination of ferrous iron is negligible, being within the experimental error of the determination. In some rocks it may exceed 0·08 per cent., as V_2O_3,[1] and for these some correction may be attempted. In order to make a correction, it is necessary to know the valency state of the vanadium. According to Goldschmidt,[9] vanadium in igneous rocks is nearly always present in the tervalent state, and for this type of rock a correction can be based on this observation. The interference is caused by the reaction—

$$V^{5+} + V^{3+} = 2V^{4+},$$

and the correction is therefore different for the two methods proposed. In the volumetric method, V^{3+} is equivalent to Fe^{2+}, whereas in the colorimetric method, since V^{4+} is equivalent to Fe^{2+}, then V^{3+} is equivalent to $2Fe^{2+}$.

In types of rocks other than igneous, such as metamorphic and sedimentary rocks, the valency state of vanadium is uncertain, as tervalent vanadium can be oxidised to vanadate during weathering; other valency states may be possible in sedimentary rocks. The value of correction is therefore doubtful.

Conclusion

Methods based on the use of quinquivalent vanadium in hydrofluoric acid and involving use of volumetric and colorimetric finishes have been developed for determining ferrous iron in silicate minerals on the micro scale. The colorimetric method is rapid and would be suitable for a large number of simultaneous determinations. Results found by both methods are in good agreement with those obtained by macro methods.

Organic carbon, pyrolusite and sulphides of iron interfere, but the interference from a sample of pyrrhotite was found to be much less than when the Pratt method was used.

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Fluorimetric Determination of Boron

Application to Silicon, Sea Water and Steel

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The reaction of borate ion with benzoin has been used for the fluorimetric determination of very small amounts of boron. Factors affecting the reaction are discussed and a high-vacuum distillation method is described for separating boron from large amounts of interfering materials. In favourable instances a simpler method of separation with an ion-exchange resin is suitable. Procedures are described for determining boron in silicon (limit of detection about 0·03 p.p.m.), in sea water (with a precision of better than ±2 per cent.) and in steel (limit of detection about 1 p.p.m.).

The fluorimetric reaction of borate ion with benzoin was first investigated by White, Weissler and Busker,[1] who applied it to determining amounts of boron in the range 0·1 to 10 μg. Subsequent investigation by the authors[2] showed that the method was capable of considerably greater sensitivity, the limit being set by the magnitude of the blank fluorescence. The method has now been refined and a general procedure has been developed for determining amounts of boron in the range 0·01 to 0·08 μg. The various factors affecting the sensitivity and detailed procedures, including that applied to determining boron in semi-conductor silicon, are described in this paper.

Choice of concentrations

The fluorescent compound is formed by the reaction of traces of borate with benzoin in alkaline aqueous-ethanolic solution. It was found that sodium hydroxide, tetramethylammonium hydroxide and sodium carbonate could equally well be used, provided that the concentration of ethanol was maintained sufficiently high. Sodium hydrogen carbonate was unsuitable. The amount of fluorescent substance formed increases as the concentration of ethanol or benzoin increases, although the observed fluorescence intensity may decrease at high concentrations of benzoin owing to an inner-filter effect.[2]

Table I

Concentrations of reagents

Reagent	Concentration in final solution, % w/w	Tolerable variation, % w/w
Ethanol	72	±0·7
Sodium carbonate	0·042	>±0·01
Benzoin	0·14	±0·02

After many trials the concentrations finally chosen were as shown in Table I. The tolerable variation in concentration corresponds to a variation of approximately 5 per cent. in the observed fluorescence intensity. The final volume was 16·5 ml (after contraction) and this was suitable for measuring boron up to 0·08 μg (*i.e.*, 0·005 μg per ml) by the prescribed procedure. The blank fluorescence corresponded to 0·003 to 0·005 μg of boron. The calibration graph was linear up to at least 0·4 μg of boron, but for these larger amounts a more concentrated standard solution of quinine bisulphate must be used.

Choice of light for excitation of fluorescence

The procedure was designed for use with a filter fluorimeter having a mercury lamp as source of exciting light. Of the principal mercury lines, those of wavenumber 2·29 μ^{-1} (436 mμ) and smaller are so little absorbed by the borate - benzoin compound that the sensitivity is too low. On the other hand, those of wavenumber 2·99 μ^{-1} (334 mμ) and greater are absorbed to such an extent by the excess of benzoin that the inner-filter effect becomes unacceptably large. The factors affecting the choice between the remaining two principal lines at 2·47 μ^{-1} (405 mμ) and 2·73 μ^{-1} (366 mμ) have been investigated previously[2,3] and can be summarised briefly as described below.

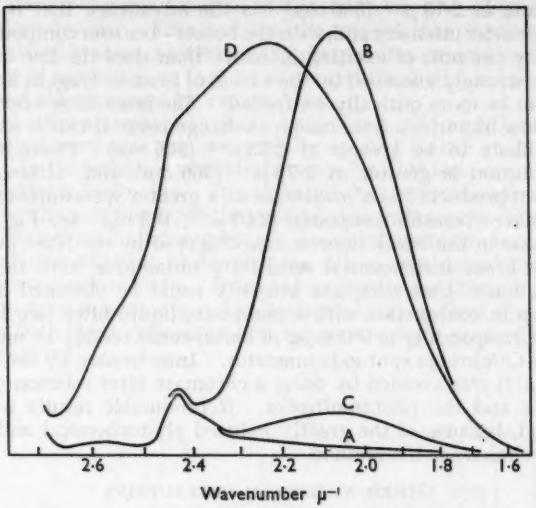


Fig. 1. Fluorescence spectra of borate - benzoin compound and benzoin photodecomposition products excited by 2·73 μ^{-1} (366 mμ) light: curve A, 0·07 μg of boron before addition of benzoin; curve B, 0·07 μg of boron in 16·5 ml of solution after reaction with benzoin; curve C, reagent blank after reaction with benzoin; curve D, solution C after photodecomposition. (Spectra are uncorrected; a quartz monochromator and 6256 B photomultiplier were used. Correction curves were given by Parker and Rees[7])

NOTE—The fluorescence emission spectrum of the borate - benzoin compound excited by 2·47 μ^{-1} (405 mμ) light has been shown by Parker.[3] It is the same as curve B except for the distortion by the Raman band of the solvent, which appears at 2·1 to 2·2 μ^{-1}. The fluorescence emission spectrum of the photoproducts from long irradiation by the 2·47 μ^{-1} mercury line is similar to curve D (apart from distortion by the Raman band)

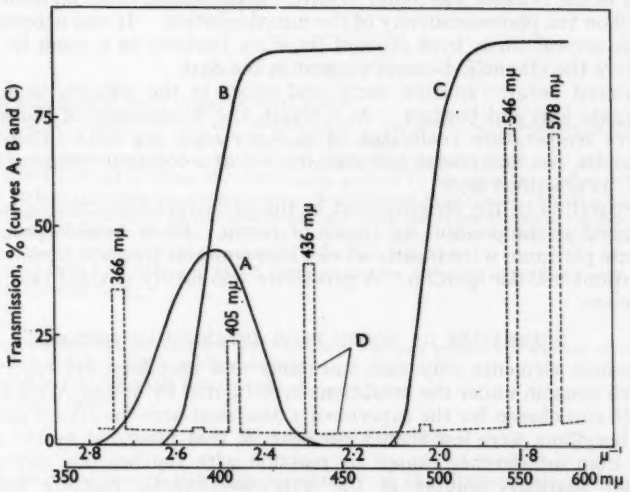


Fig. 2. Transmission curves of primary and secondary filter cells: curve A, 1 cm of a 0·75 per cent. solution of iodine in carbon tetrachloride and 1 cm of a solution containing 100 g of $Cu(NO_3)_2.3H_2O$ in 60 ml of *N* nitric acid; curve B, 1·5 cm of a 1 per cent. w/v aqueous solution of sodium nitrite; curve C, 1·5 cm of a 1 per cent. w/v solution of potassium chromate in 0·05 per cent. w/v sodium hydroxide; curve D, approximate spectral distribution of light from mercury lamp (quanta)

The mercury line at 2·73 μ^{-1} (366 mμ) has the advantage that it is emitted by most mercury lamps at a greater intensity and, with the borate - benzoin compound, it gives a greater fluorescence intensity per unit of exciting intensity than does the line at 2·47 μ^{-1} (405 mμ). It is, however, more strongly absorbed by the excess of benzoin reagent and the concentration of this reagent has to be more critically controlled. The inner-filter effect due to other trace impurities (*e.g.*, yellow impurities from cation-exchange resin, if this is used in the separation procedure) is also likely to be greater at 2·73 μ^{-1} (366 mμ). Photodecomposition of the alkaline benzoin solution is greater at 2·73 μ^{-1} (366 mμ) and, although the fluorescence spectrum of the photoproducts has a maximum at a greater wavenumber (2·22 μ^{-1}; 450 mμ) than that of the borate - benzoin compound (2·07 μ^{-1}; 483 mμ—see Fig. 1), it still produces a considerable increase in the blank fluorescence observed by the filter fluorimeter.

In spite of the lower instrumental sensitivity obtainable with the 2·47 μ^{-1} (405 mμ) mercury line, it was found that adequate intensity could be obtained by using a 250-watt compact-source lamp in conjunction with a composite liquid filter (see Fig. 2, curves A and B). Fluorescence corresponding to 0·005 μg of boron could readily be measured with a 931A photomultiplier and Cambridge spot galvanometer. Interference by the Raman band of the solvent[3] (2·1 to 2·2 μ^{-1}) was avoided by using a chromate filter solution (see Fig. 2, curve C) between the cuvette and the photomultiplier. Reproducible results were obtained under these conditions, and, because of the greatly reduced photochemical and inner-filter effects, this line was finally chosen for the method.

Other necessary precautions

Oxygen produces the undesirable effects mentioned below, even when present at low concentration[2]—

(*a*) Reversible quenching of the borate - benzoin fluorescence and slow irreversible decomposition.

(*b*) Oxidation of the benzoin to produce absorbing products.

(*c*) Acceleration of the photodecomposition of the benzoin reagent.

To obtain reproducibly low blank values it was found necessary to de-oxygenate completely both the alkaline borate solution and the ethanolic benzoin solution before mixing them; a special reaction cell was used.[2]

The purity of the benzoin was found to have a considerable effect on the magnitude of the blank value and on the photosensitivity of the mixed solution. It was necessary to recrystallise the benzoin several times from ethanol (in silica beakers) in a room lit only by yellow light, and to store the ethanolic benzoin reagent in the dark.

The fluorescent borate - benzoin compound exists in the solution in equilibrium with uncombined borate ions and benzoin. As a result, the fluorescence of the solution shows a marked negative temperature coefficient (3 to 4 per cent. per °C).[2] Therefore, to obtain reproducible results, the fluorimeter cell was situated in a constant-temperature block maintained at 20° C, as described later.

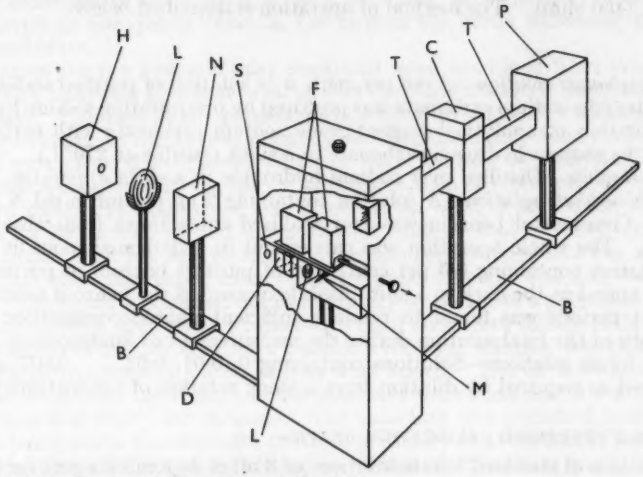
In the preparation of the reagents and in the procedure the strictest precautions were necessary to avoid contamination by traces of boron. Much trouble was experienced in attempting to use platinum ware (nearly all of which contains traces of boron) for evaporation of alkaline solutions and for ignition. A procedure was finally devised that did not require the use of platinum.

Separation of boron from interfering materials

Of the common elements only zinc, antimony and beryllium are reported to produce fluorescence with benzoin under the conditions investigated by White, Weissler and Busker.[1] Under the conditions chosen for the experiments described here the effects produced by zinc, antimony and beryllium were less than 1 per cent. of that produced by the same weight of boron. Apart from interference caused by reaction with the benzoin, any substance (*e.g.*, sodium chloride) sparingly soluble in the aqueous-ethanolic mixture will interfere, if present in sufficient quantity, by producing a precipitate in the fluorimeter cell. This source of interference could be removed by distillation of the boron. Distillation as methyl borate has been used by many previous workers. As normally applied, it suffers from the disadvantage that comparatively large volumes of distillate have to be collected and then evaporated in the presence of a comparatively large amount of alkali. Experience showed

that there was great danger of contamination in this procedure and a high-vacuum distillation procedure was therefore devised. This had the advantages that the distillation was carried out in a completely closed system, so minimising danger of contamination, and that ethanol could be used in place of methanol, so that an aliquot of the distillate could be taken directly for fluorimetry without the necessity for evaporation.

In the absence of large amounts of volatile anions (*e.g.*, Cl^- or NO_3^-) this procedure was found to be very satisfactory and was successfully applied to the analysis of silicon, fused magnesia and steel. In the presence of chloride, some hydrochloric acid is distilled and must be neutralised with sodium hydroxide. It was found that approximate results could still be obtained by decanting the solution from the precipitated sodium chloride before fluorimetry. The application to high-purity silicon presents a special problem, because the amount of sodium hydroxide solution required to dissolve the silicon at atmospheric pressure gives rise to an unacceptably high blank reading. In addition, distillation from large amounts of silicic acid was found to be very incomplete. The hydrothermal method described by Luke and Flaschen[4] overcomes both these difficulties and was successfully applied before high-vacuum distillation and fluorimetry.



B = Steel bars fixed at right angles to each other
C = Housing for 1·5-cm optical cell containing a 1 per cent. w/v solution of potassium chromate in 0·05 per cent. w/v sodium hydroxide
D = Sealed double-compartment optical cell containing copper nitrate solution (100 g of $Cu(NO_3)_2.3H_2O$ in 60 ml of *N* nitric acid) and a 0·75 per cent. w/v solution of iodine in carbon tetrachloride, respectively. Both optical depths are approximately 1 cm
F = Fluorimeter cuvettes in constant-temperature block (both are shown as simple cuvettes, although one, the reaction cell, was of special construction as previously described[2]). For clarity the constant temperature block is not shown
H = Housing of G.E.C. 250-watt ME/D compact-source mercury lamp fitted with Chance ON2O heat filter
L = Lenses
M = Mirror
N = 1·5-cm optical cell containing 1 per cent. w/v aqueous solution of sodium nitrite
P = Housing of R.C.A. 931A photomultiplier
S = Shutter in primary beam (not visible)
T = Light-tight tubes

Fig. 3. Diagram of fluorimeter

Advantage can be taken of the high sensitivity of the fluorimetric method (0·002 μg of boron in 3 ml of water) to determine boron at the part-per-million level in some materials without distillation. For example, in the analysis of sea water it was necessary to take the equivalent of only 0·01 ml in the fluorimeter cell; the sodium chloride contained in this small amount was not sufficient to interfere. A simple procedure was thus used, involving treatment with an ion-exchange resin and neutralisation before an aliquot was taken for fluorimetry.

Method

Fluorimeter—

A simple filter fluorimeter was constructed specifically for determining boron; it is shown diagrammatically in Fig. 3. The fluorimeter was built round the fluorescence attachment of a Spekker absorptiometer. The normal cell holder and housing was replaced by a constant-temperature block holding the special fluorimeter cell (of fused quartz) and a rectangular Spekker glass fluorimeter cell holding the standard quinine bisulphate solution (2 μg per ml in 0·1 *N* sulphuric acid). The constant-temperature block and special fluorimeter cell with trap have been described previously.[2] Light from the mercury lamp (250-watt compact source) was passed via the glass heat filter (Chance ON20) and lens to the composite liquid filter for isolation of the 2·47 μ^{-1} (405 mμ) mercury line (see Fig. 2, curves A and B). The monochromatic beam passed into the Spekker attachment in which it was reflected vertically upwards through the base of one of the fluorimeter cells. The resulting fluorescence was viewed through the side of the cell via the secondary chromate filter (see Fig. 2, curve C) by an R.C.A. 931A photomultiplier. The latter was operated by a commercial stabilised 1000-volt supply and the photomultiplier output was detected by means of a Cambridge spot galvanometer (450 ohm). The method of operation is described below.

Reagents—

Sodium carbonate solution—A 0·6 per cent. w/v solution of purified sodium carbonate in de-ionised water (the sodium carbonate was prepared by precipitating sodium hydrogen carbonate from a solution of analytical-reagent grade sodium carbonate with carbon dioxide and then igniting the sodium hydrogen carbonate in a silica crucible at 270° C).

Ethanol, absolute—Distilled over sodium hydroxide in a silica apparatus.

Quinine bisulphate solution—A solution containing 2 μg per ml in 0·1 *N* sulphuric acid.

Benzoin—Commercial benzoin was recrystallised three times from absolute ethanol in silica beakers. The whole operation was carried out in a darkened room lit by yellow safe lights. A solution containing 0·5 per cent. w/v of purified benzoin in purified ethanol was prepared and stored in the dark in a silica flask (exposure of the benzoin solution to daylight even for short periods was found to produce sufficient photodecomposition to cause high photosensitivity of the final solution during the measurement of fluorescence).

Standard borate solutions—Solutions containing 0, 0·01, 0·02 . . . 0·07 μg of boron per 3 ml. Prepared as required by dilution from a stock solution of concentrated boric acid.

Procedure for preparing calibration graph—

A 3-ml portion of standard borate solution (or 3 ml of de-ionised water for the blank test), 1 ml of 0·6 per cent. sodium carbonate solution and 9 ml of absolute ethanol were placed in the fluorescence compartment of the special cell by means of silica pipettes. The trap and de-oxygenating tubes were assembled and the cell was placed in the constant-temperature block of the fluorimeter. A 4-ml portion of 0·5 per cent. benzoin solution was placed by means of a pipette in the trap and the flow of nitrogen (B.O.C. "white spot") was begun and maintained at 30 ml per minute for 20 minutes. Towards the end of this period, the "stray" fluorescence (caused by trace impurities in the ethanol or sample, etc.) was measured as follows. With the standard cell (containing 17 ml of the quinine bisulphate solution) in the beam, the shutter was opened and the galvanometer adjusted to full-scale deflection by variation of the photomultiplier voltage and galvanometer shunt setting. The borate cell was then moved into the beam and the small galvanometer deflection, corresponding to the "stray" fluorescence, was noted. After de-aeration, the benzoin reagent in the trap was allowed to flow into the borate solution, and, after reaction for 10 minutes with nitrogen still passing, the fluorescence intensity was again measured by reference to the quinine standard. The "reading" corresponding to the "stray" fluorescence was deducted from the reading observed after reaction with the benzoin, giving the net galvanometer reading corresponding to the total borate present in the cell. A similar net reading corresponding to the test in which no borate was added (*i.e.*, the boron reagent blank value) was deducted and the final readings were plotted against micrograms of boron added. A linear calibration graph was obtained.

Full-scale deflection corresponded to the addition of about 0·08 μg of boron. The "stray" fluorescence was small under normal conditions (equivalent to 0·004 μg of boron). If the

"stray" fluorescence exceeded 0·006 μg the result was rejected and the water and ethanol were re-purified. The net boron blank reading corresponded to about 0·004 μg of boron. Results in excess of this figure indicated insufficiently purified reagents. The most frequent cause of high blank values was found to be either traces of oxygen (owing to insufficient de-aeration) or exposure of the benzoin reagent to light, the symptom for both causes being a steadily increasing fluorescence reading during exposure to the light in the fluorimeter.

PROCEDURE FOR DETERMINING BORON IN HIGH-PURITY SILICON—

The sample was crushed in a steel percussion mortar and passed through a B.S. No. 72 brass sieve. The sifted sample was cleaned by boiling in two successive portions of analytical-reagent grade concentrated hydrochloric acid and washing free from chloride with de-ionised water. It was then dried at 130° C and weighed.

The boron was separated by reaction of the powdered silicon with dilute sodium hydroxide solution in a small autoclave at 350° C and a pressure of about 350 atmospheres (the hydrothermal method as used by Luke and Flaschen[4]). The amount of sodium hydroxide is much less than that required to convert the silicon to sodium silicate and the effective over-all reaction is the oxidation of the silicon to silica by water, with the liberation of hydrogen. The silica separates as fine quartz crystals, the bulk of the boron remaining in the alkaline mother liquor as borate.

The autoclaves for the hydrothermal treatment were machined from mild steel to the design proposed by Luke and Flaschen,[4] except that the plunger was left undrilled and no metal liner was inserted. The face of the plunger was polished to prevent it sticking to the silver cover disc. The volume of the autoclave cavity was approximately 30 ml. Before each test the autoclaves were cleaned as follows: 20 ml of water and 5 g of analytical-reagent grade sodium hydroxide were placed in the cavity, which was then covered with the silver disc and the top screwed tight. The autoclave was placed in an oven at 350° C for 2 hours; it was then quenched for 30 seconds in cold water and set aside to cool to room temperature in a shallow water bath. The top was unscrewed, the plunger removed and the shoulder of the autoclave and the silver disc wiped clean with dry paper tissue. The disc was then pierced and removed. The liquid was sucked out of the cavity by means of a polythene tube attached to a water pump and the cavity was washed with eight successive fillings of de-ionised water.

A 20-ml portion of 0·2 per cent. w/v sodium hydroxide solution was transferred to the cavity and 1·5 g of the purified silicon sample were added. The cavity was covered with a fresh boron-free silver disc, the threads of the autoclave were oiled, and it was then assembled as before and heated at 350° C for 16 hours. The autoclave was quenched, cooled and opened as before, care being taken to relieve the pressure gradually to avoid loss of liquid. The bulk of the supernatant liquid was removed from the residue of crystalline quartz and stored in a closed silica flask.

Ten millilitres of the solution from the autoclave were transferred to flask A of the distillation apparatus (see Fig. 4). The apparatus was assembled and tap T was closed. The flask was immersed in liquid nitrogen for 5 minutes, the tap was opened, and the still was then pumped down to high vacuum (less than 10^{-4} mm of mercury). Tap T was closed, the liquid-nitrogen bath was transferred from flask A to flask B, and flask A was immersed in water at 60° C. Distillation was allowed to continue until the volume remaining had been reduced to 2 ml. The liquid-nitrogen bath was removed, the apparatus was opened to the atmosphere, flask A was dismantled and 0·25 ml of 60 per cent. perchloric acid and 10 ml of absolute ethanol were added, with swirling to mix completely. The apparatus was re-assembled and distillation continued as before, after appropriate freezing and pumping, etc., until about 0·5 ml of liquid remained in flask A. A second distillation was then carried out after the addition of a further 10 ml of absolute ethanol to the residue in flask A. This last distillation was continued to dryness. Altogether, 10 ml of water and 20 ml of absolute ethanol were thus collected in the receiver. One fifth of this solution was then transferred to the fluorimeter cell, 2 ml of 0·3 per cent. sodium carbonate solution and 5 ml of absolute ethanol were added, and the fluorimetric procedure was carried out as described above. A blank test on the same autoclave was carried through the whole procedure, omitting only the silicon (the aliquot taken for the blank determination was increased from 10·0 to 11·1 ml—see "Method for Pure Silicon," p. 835).

The results of recovery tests in which the procedure was applied to portions of a sample of pure silicon and in which known amounts of borate had been added to the autoclave liquid

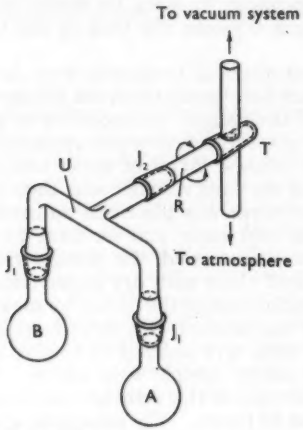
before heating are shown in Table III (see p. 836) and discussed below. The results obtained on samples of silicon containing different amounts of boron are shown in Table IV (see p. 837).

PROCEDURE FOR DETERMINING BORATE IN SEA WATER—

Twenty millilitres of sea water were diluted to 200 ml with de-ionised water. Four 10-ml portions of this solution were passed through a cation-exchange column (Amberlite IR-120 in acid form) 45 cm long and approximately 1·3 cm in diameter. The effluent was discarded and a further 60 ml of the solution were passed through the column, from which the last 30 ml of effluent were collected for the test. A 5-ml portion of this solution was titrated with 0·1 *N* sodium hydroxide, phenolphthalein being used as indicator.

To a second 5-ml portion was added the equivalent volume of sodium hydroxide solution and the mixture was diluted to 100 ml with de-ionised water; 3 ml of this solution were transferred to the fluorimeter cuvette and the fluorimetric procedure was carried out as described under "Procedure for Preparing Calibration Graph," p. 832.

Results obtained with samples of artificial sea water[5] and natural sea water are discussed below.



A, B = 50-ml silica flasks
J_1 = Standard B24 joints sealed with polytetrafluoroethylene sleeves
J_2 = Standard B14 joint sealed with vacuum grease
R = Silica tube rotated to close tap T
T = 3-way tap sealed with vacuum grease
U = Connecting tube and side-arm of silica

Fig. 4. High-vacuum still

PROCEDURE FOR DETERMINING BORON IN STEEL—

One piece of sample (approximately 1 g) was etched for a few minutes in hot concentrated nitric acid, washed with water, dried and weighed. It was then dissolved by boiling under reflux in all-silica apparatus with 12 ml of 5 *N* sulphuric acid. When the solution had cooled, 1 ml of 50 per cent. w/v redistilled hydrogen peroxide was added to oxidise the iron and the suspension of carbon. The excess of peroxide was destroyed by boiling under reflux for 30 minutes, and the solution was filtered through a small acid-washed filter-paper (Whatman No. 44), the filtrate being collected in a 100-ml calibrated flask. The reaction flask was washed several times with 0·1 *N* sulphuric acid, the washings being passed through the filter-paper, which was then washed three times more with the same acid. All the washings were collected in the calibrated flask and made up to volume at 20° C with water. The filter-paper was then washed with ethanol and dried. The filter-paper was fixed in a small platinum-gauze basket and burned in oxygen in a stoppered 500-ml silica flask by the method described by Corner.[6] The contents of the calibrated flask were poured into the cooled combustion flask and shaken to dissolve any residue. Ten millilitres of the resulting solution were transferred to flask A of the distillation apparatus, and the borate was distilled as described under "Procedure for

Determining Boron in High-purity Silicon," p. 833, except that no perchloric acid was added. An appropriate aliquot of the distillate was taken for fluorimetric determination, the ethanol - water ratio being adjusted as necessary.

The reagent blank value was determined by carrying out the same procedure with a 30-mg sample of Specpure iron (added only to assist the decomposition of the excess of hydrogen peroxide), the excess of sulphuric acid (*i.e.*, equivalent to approximately 1 g of iron) being neutralised with 2 g of sodium hydroxide of known boron content (0·06 p.p.m.—determined fluorimetrically after treatment with ion-exchange resin and distillation).

Sensitivity and precision of methods

Direct method—

As with most methods of trace analysis the sensitivity was limited by the magnitude and reproducibility of the reagent blank value, which, in turn, varied according to the complexity of the chemical operations required to separate the boron from the particular material being analysed. The simple fluorimetric procedure (*i.e.*, as described under "Procedure for Preparing Calibration Graph," which is applicable to aqueous solutions containing salts only up to a limit governed by solubility in the final aqueous-ethanolic solution) was applicable to amounts of boron up to 0·08 μg in the 3 ml of aqueous solution taken. The blank value varied between 0·003 and 0·005 μg (5 to 8 galvanometer-scale divisions) according to the batch of reagents used. With the same batch of reagents the blank value was reproducible to ±0·0005 μg of boron. The minimum significant amount of boron in excess of the blank reading was thus assumed to be 0·002 μg. At the upper end of the scale, the reproducibility can be judged from a typical set of results observed for five separate 0·07-μg portions of boron, namely: 112, 112, 110, 108 and 108 galvanometer-scale divisions, *i.e.*, a spread of ±0·0013 μg on 0·07 μg.

Distillation methods—

Only one-fifth of the boron distilled was taken for fluorimetry and the resulting sensitivity was correspondingly reduced. The sensitivity and reproducibility of the distillation procedure can be judged from the results shown in Table II. The deviations from theoretical recovery were all within +0·012 and −0·014 μg. On the basis of these figures and the reproducibility of the blank value, the minimum weight of boron detectable by the distillation method was assumed to be about 0·01 μg.

Table II

Recovery of boron after high-vacuum distillation

Ten millilitres of a 0·2 per cent. w/v solution of sodium hydroxide containing the boron were placed in the distillation flask. The direct fluorimetric blank value (0·003 μg of boron), calculated in terms of boron distilled, was 0·015 μg. Ten distillations carried out with no added boron gave values within the range 0·020 ± 0·003 μg

Boron added, μg	Boron found,* μg	Boron added, μg	Boron found,* μg
0·027	0·020	0·188	0·174
0·054	0·046	0·215	0·216
0·081	0·084	0·242	0·228
0·108	0·094	0·269	0·254
0·135	0·128	0·296	0·292
0·161	0·152	0·323	0·314
		0·350	0·362

* Results corrected for blank value of 0·020 μg.

Method for pure silicon—

The method involved the reaction of 1·5 g of silicon with 20 ml of 0·2 per cent. w/v sodium hydroxide in the autoclave. Owing to the reaction

$$Si + 2H_2O \longrightarrow SiO_2 + 2H_2$$

1·9 ml of water were consumed, and the boron was thus concentrated in the proportion $\frac{20}{18{\cdot}1}$. A 10-ml portion of the mother liquor was taken for distillation and this aliquot thus corresponded to 0·83 g of silicon. However, to determine the blank value due to boron either

present in the sodium hydroxide solution or extracted from the autoclave during reaction, tests were carried out in which the silicon was omitted. To allow for the fact that concentration of the solution did not take place in the absence of silicon, the aliquot taken for distillation in the tests was increased from 10·0 to 11·1 ml, the quantity of ethanol being adjusted accordingly. The amounts of boron recovered from six such blank tests (after correction for the fluorimetric blank—0·02 μg in this instance) were 0·017, 0·020, 0·013, 0·023, 0·013 and 0·013 μg. The mean value of 0·016 μg, calculated on 0·83 g of silicon, thus corresponded to 0·019 p.p.m.

The mother liquor from the autoclave after crystallisation of the quartz contained appreciable amounts of silica, and it was expected that the residue after distillation of the ethanol might retain an appreciable proportion of the boron present. Experiments in which borate was added to the mother liquor from the autoclave before distillation showed that losses up to 10 per cent. could occur due to this cause. To correct for this and also for possible loss of borate by adsorption in the quartz crystals during reaction, a fresh calibration curve was prepared from the results on 1·5-g portions of a sample of pure silicon in the presence of different amounts of added borate. Many tests were also made on the same sample of silicon, but with no added borate; the results are shown in Table III. Recoveries varied from 82 to 91

TABLE III

RECOVERY OF BORATE FROM SILICON REACTION MIXTURE

Boron added, p.p.m.	Boron found, p.p.m.	Recovery (after correction for boron in silicon), %
Nil	−0·006, nil, 0·001, 0·001, 0·006, 0·007, 0·008, 0·011, 0·013, 0·013, 0·018, 0·018, 0·020, 0·020, 0·020, 0·024 (Mean, 0·011)	—
0·088	0·072	82
	0·080	91
0·220	0·181	82
	0·188	85
0·308	0·280	91
	0·272	88
0·440	0·375	85
	0·381	87

NOTE—(*a*) One microgram of boron added in the autoclave corresponds to 0·67 p.p.m. of boron in the silicon sample.
(*b*) The borate was added before reaction in the autoclave.
(*c*) The results on the pure silicon provide a measure of the sensitivity of the method. The root mean square deviation from the mean is 0·009; thus the 95 per cent. confidence value is $\pm$0·018 for a single determination, $\pm$0·009 for the mean of four determinations and $\pm$0·0045 for the mean of sixteen determinations. The boron content of the sample used for obtaining the results in the Table is therefore 0·011 $\pm$ 0·005 p.p.m.

per cent. The best calibration curve drawn through the points had a slope that was 86 per cent. of that obtained by direct fluorimetric reaction. This new calibration curve was used for the analysis of the samples of silicon for which the results are shown in Table IV. The nominal boron contents of these samples were known only approximately. The samples had been prepared by "doping" pure silicon with boron and growing a single crystal to make a "master" standard containing 30 p.p.m. of boron. This was then diluted successively with pure silicon to prepare single crystals of lower boron content. The results indicate that the method provides a reasonably reliable estimate of boron content down to about 0·08 p.p.m. Provided that several determinations are made, detection is reasonably reliable down to about 0·03 p.p.m.

METHOD FOR SEA WATER—

Sea water contains about 5 mg of boron (as borate) per litre. It is normally determined by titration of 100 ml of sea water with dilute sodium hydroxide solution after addition of mannitol, with phenolphthalein as indicator. The end-point of this titration is somewhat

TABLE IV

DETERMINATION OF BORON IN "BORON-DOPED" SILICON CRYSTALS

Nominal boron content, p.p.m.	Boron found in individual determinations, p.p.m.	Mean, p.p.m.
0·003	−0·005, 0·006, −0·001, 0·006, −0·001, 0·010, Nil, 0·022	0·005
0·008	0·019, 0·026, 0·029	0·025
0·025	0·027, 0·034, 0·034, 0·039	0·034
0·083	0·060, 0·060, 0·069, 0·069, 0·100	0·070
0·25	0·19, 0·19, 0·21	0·20
0·86	0·76, 0·83, 0·86	0·82
2·6	1·5	1·5
9	9·5	9·5

difficult to judge with accuracy. The fluorimetric method is more convenient once the apparatus has been set up and can be applied to much smaller samples. Its reliability was first tested with samples of artificial sea water made up according to the specification quoted by Harvey,[5] but with no borate added. In five tests, the amounts of boron found were between 0·04 and 0·08 mg per litre. This presumably corresponds to traces of boron present in the salts used. To a further volume of the artificial sea water was added borate equivalent to 5·10 mg per litre. Results for the determination of boron by reference to the standard fluorimetric calibration graph were 4·92, 4·92, 4·96, 5·00 and 5·04 mg per litre. The mean value is about 4 per cent. low. This is attributed to the effect of the sodium salts on the dissociation of the borate - benzoin compound. The effect was confirmed by preparing a new calibration graph with the artificial sea water to which had been added varying amounts of borate. This calibration graph was then used in the determination of the boron contents of the sea water samples shown in Table V. The reproducibility for a single determination is better than ±0·1 p.p.m.

TABLE V

DETERMINATION OF BORATE IN SEA WATER

Source	Boron content, mg per litre	Mean, mg per litre	Salinity	Ratio of boron content to salinity
English Channel (Portland, October, 1959)	4·74, 4·78, 4·83, 4·87, 4·87	4·82	34·8	0·139
English Channel (Selsey, April, 1959)	5·14, 5·14, 5·18, 5·23	5·17	38·3	0·135
Arctic (Northern Norwegian Sea, May, 1959)	4·13, 4·17, 4·17, 4·17, 4·21	4·17	31·7	0·132
Red Sea (near mouth of Gulf of Suez, October, 1958)	5·40, 5·48, 5·48, 5·48, 5·53	5·47	39·7	0·138

METHOD FOR MILD STEEL—

The method is satisfactory for levels at which small amounts of boron are normally required to be determined in steel (*i.e.*, about 5 p.p.m. and greater), as indicated by the results

shown in Table VI. With the procedure used, somewhat lower boron contents could have been determined, a limit of about 1 p.p.m. being set by the magnitude of the reagent blank value ($1{\cdot}3 \pm 0{\cdot}2$ p.p.m.). The magnitude of the blank value is much greater than the potential sensitivity of the fluorimetric procedure, and, if required, a further gain in sensitivity could probably be achieved by purification of the sulphuric acid and the filter-paper.

Table VI

Determination of boron in steel

Sample	Nominal boron content, p.p.m.	Boron found, p.p.m.
Standard boron steel	190	196
	87	80
	27	23
	6	6
Autoclave steel (medium carbon)	—	<1
Spectrographic iron electrode	—	<1

Conclusions

(*a*) In the absence of interfering elements, direct fluorimetry provides a rapid method for determining boron in the range 0·01 to 0·08 μg. The limiting sensitivity is 0·002 μg.

(*b*) Traces of borate can be separated quantitatively from large amounts of ions forming ethanol-soluble perchlorates (*e.g.*, sodium and magnesium) or sulphates (*e.g.*, iron) by high-vacuum distillation. The limiting sensitivity is about 0·01 μg of boron.

(*c*) Boron in silicon can be determined down to less than 0·1 p.p.m. The limiting sensitivity is about 0·03 p.p.m.

(*d*) The fluorimetric method for sea water is more reliable than the conventional method and can, if necessary, be applied to very small samples (0·01 ml).

(*e*) Boron can be determined satisfactorily in steel containing 5 p.p.m. or more. The limiting sensitivity (about 1 p.p.m.) is governed by the magnitude of the reagent blank value. It could probably be reduced considerably by purification of the reagents and materials.

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Notes

THE COMPOSITION OF SOME NEW ZEALAND SKIMMED-MILK POWDER AND ITS INFLUENCE ON THE DETERMINATION OF MILK-SOLIDS-NOT-FAT

AFTER examining many samples of liquid milk in the years 1881 to 1888, Vieth[1,2] noticed that the proportions of ash, protein and anhydrous lactose were approximately in the ratio 1 to 5 to 6; subsequently,[3] he stated that the ratio 2 to 9 to 13 was more exact. These later figures are quoted in standard text-books[4,5] and are used as a basis for calculating the amount of non-fatty milk solids in ice-cream and dairy products. In the absence of interfering substances, non-fatty milk solids are derived by multiplying the anhydrous lactose content by 24/13 or the protein content by 24/9.

When we applied this principle to the calculation of non-fatty milk solids in ice-cream samples of known and unknown composition, the results obtained by using the two factors generally disagreed, and, as New Zealand milk powder was an ingredient of most of the samples, twenty-two such powders were examined. The samples were taken at random from different deliveries in the period April until September, 1959, and information on the time and place of manufacture and the breed of cow was not available. Fifteen powders were spray-dried, the remainder were roller-dried. Lactose was determined by Lane and Eynon's method[6] after clarification with zinc acetate and potassium ferrocyanide solutions[7]; protein was determined by the Kjeldahl method.

RESULTS

The Vieth ratios for nineteen of these powders were—

Anhydrous lactose	11·70 (standard deviation 0·14)
Protein	10·25 (standard deviation 0·15)
Ash	2·05 (standard deviation 0·05)

and for the remaining three powders, the ratios were—

Anhydrous lactose	10·60 (standard deviation 0·16)
Protein	11·40 (standard deviation 0·14)
Ash	2·00 (standard deviation 0·08)

The results of an analysis of a milk powder in the second group are shown below.

Lactose monohydrate, %	43·1
Protein (N × 6·38), %	43·3
Ash, %	7·7
Moisture, %	3·7
Fat, %	1·1
Acidity, as lactic acid, %	1·0
Casein, %	36·4

It is clear that the commonly accepted values of the Vieth ratio do not apply to these New Zealand skimmed-milk powders, and for most of these products, Vieth's original ratio is much nearer the truth. However, a small proportion of powders was abnormal. In the routine examination of ice-cream manufactured from New Zealand milk powder it seems reasonable to take a weighted average of the values found; this gives a ratio of anhydrous lactose to protein to ash of 11·55 to 10·40 to 2·05.

COMPOSITION OF NON-FATTY MILK SOLIDS

If these values are used instead of the commonly accepted values of the Vieth ratio, the factors for calculating non-fatty milk solids from lactose or protein become 24/11·55 or 24/10·40, respectively. The figure derived when these factors are used is the sum of the anhydrous lactose, protein and ash. It therefore excludes minor constituents, such as citrates, which should be included, and also ignores water of hydration present in the lactose.

Recently published evidence[8] states that the water of crystallisation of hydrated lactose is lost slowly at 100° C. This contradicts earlier evidence,[9,10] which also states that, when solutions of lactose and milk are evaporated to dryness by heating on a bath of briskly boiling water, with frequent stirring, the residue is anhydrous lactose. It is pointed out, however, that the removal of water of hydration is not complete. More recently, it has been stated[11] that hydrated lactose crystallises from water below 94° C, whereas anhydrous lactose crystallises above this temperature. Since the temperature of the contents of a dish on a bath of boiling water is usually less than 94° C, hydrated lactose should be formed.

In order to resolve these contradictions, hydrated lactose was dried for up to 7 hours in both an air oven and a vacuum oven regulated at 100° C. In neither instance was any loss in weight detected, and chemical assay of the residues confirmed them to be hydrated lactose. Also, when solutions containing known amounts of dried hydrated lactose were dried on the water bath and in the air oven, no loss in the original weight of the lactose was detected. It was concluded that the lactose was present in the hydrated form, and chemical assay again confirmed this. This drying experiment was repeated with spray-dried skimmed-milk powder; only a slight loss in weight (0·06 per cent.) was recorded.

For these reasons, we consider that the term "non-fatty milk solids" should include the water of hydration of the lactose. By defining non-fatty milk solids in milk powder to be 100 — (percentage of fat + percentage of moisture), it was found that, for the twenty-two milk powders examined, the proportions of non-fatty milk solids, anhydrous lactose, protein and ash were in the ratio 25·25 to 11·55 to 10·40 to 2·05. This relationship has been applied successfully over a long period in the determination of non-fatty milk solids in samples of ice-cream containing New Zealand milk powder. A single sample of English spray-dried milk powder gave a corresponding ratio of 24·8 to 12·2 to 9·8 to 2·0.

Conclusion

In the absence of interfering substances, non-fatty milk solids in ice-cream and other dairy products containing New Zealand skimmed-milk powder can be calculated by multiplying the anhydrous lactose content by 25·25/11·55, *i.e.*, 2·18, or the protein content by 25·25/10·40, *i.e.*, 2·43.

We thank the Boards of Unilever Ltd. and T. Wall & Sons (Ice Cream) Ltd. for permission to publish this Note.

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A MODIFIED CAVETT METHOD FOR THE DETERMINATION OF ALCOHOL IN BODY FLUIDS

In 1954, Kent-Jones and Taylor[1] reported the results of the Analytical Committee of the British Medical Association appointed to consider methods of determining alcohol in body fluids. Two methods were considered in detail; it was found that the micro Cavett method was the more accurate, but that Kozelka and Hine's method was the more precise in reproducibility. The Cavett method, which is simple and labour-saving, suffers from the defect that traces of foreign matter may affect the results, and, in practice, major and unpredictable inaccuracies arose in some laboratories. For this reason, we in the Metropolitan Police laboratory adhered to the more arduous and costly Kozelka and Hine method. It became obvious that the increasing volume of work would overload the capacity of the laboratory if this method was used, and, accordingly, the Cavett method was investigated in an effort to improve its reproducibility. The main causes of error in the original method are causes inherent in micro methods and render such methods somewhat unsuitable for use in a routine laboratory performing large numbers of analyses. Attempts were made to adapt the method as a macro method, but it was found that, under these conditions, the end-point of the titration with the "red fluid" was obscured by the chromium colour. It was

found that titration with sodium thiosulphate solution in presence of potassium iodide gave satisfactory and consistent results, so this was adopted in place of titration with "red fluid."

Preliminary experiments showed that a solution of potassium dichromate in 50 per cent. v/v aqueous sulphuric acid completely absorbed and oxidised the alcohol in 2 ml of urine or blood in 1 hour at 70° C, 8 hours at 37° C and in a variable time (12 to 24 hours) at room temperature. The most suitable temperature for the test was chosen as being 37° C. Since this temperature is arbitrary and the results are not affected by comparatively large variations, it is not necessary to use an elaborate thermostat; any insulated cabinet heated to about 37° C is suitable. We use an inoperative incubator, inside which is suspended a 100-watt electric-light bulb that maintains a temperature of 35° to 38° C day and night without attention. In place of the Cavett unit, a wide-mouthed squat 16-ounce specimen jar with a metal screw-cap was adopted as being cheap and readily available. The dichromate solution is placed in the jar, and the body fluid is measured into a 5- to 6-cm Petri dish supported in the jar by a glass triangle on 1- to 1¼-inch glass legs.

When these conditions were used, we found that variable and significant losses occurred, which were traced to "breathing" of the apparatus through the waxed-paper washer while warming in the incubator. To prevent this, the mouths of the jars were ground and a circular ground-glass plate was cut to fit the cap, the paper washer being retained to prevent strains on the plate. When this modified procedure was used, it was found that the precision of the method was equal to that of the original Cavett method and the reproducibility was better even than that of the Kozelka and Hine method and appeared to be limited only by the accuracy of measurement of the various liquids used. Records of unselected consecutive duplicate determinations by ten operators of dissimilar experience and skill showed the degree of variance given below.

No. of determinations (duplicates) ..	69	54	60	33	4	14
Difference between duplicate titres, ml ..	0·0	0·05	0·10	0·15	0·20	> 0·20
Difference, as mg of ethanol per 100 ml of body fluid	0	3	6	8·5	12	> 12

Although these results, as they were all obtained in one laboratory, may not be strictly comparable, this degree of variance is nevertheless superior to those shown in the original methods, and the ease and economy of the method render it invaluable in a laboratory handling large numbers of samples.

It should be borne in mind that, as in the Cavett and all similar methods, the presence of any volatile reducing substance affects the results, and care must be taken not only to ensure the absolute cleanliness of the apparatus, but to ascertain whether or not acetone, ether, salicylic acid, paraldehyde, etc., are present, and suitable precautions must be taken.

Method

Apparatus—

Jars—Wide-mouthed squat 16-ounce specimen jars, with ground tops and screw-caps; the caps are fitted with circular ground-glass plates. Jars and plates can be obtained from Aimer Products Ltd., Camden Road, London.

Glass triangles supported by 1- to 1¼-inch legs.

Petri dishes—5 to 6 cm in diameter.

Incubator or cabinet—Temperature controlled to approximately 37° C.

Reagents—

Potassium dichromate solution—Dissolve 4·9 g of potassium dichromate in 1 litre of 50 per cent. v/v sulphuric acid.

Potassium iodide—Analytical-reagent grade.

Sodium thiosulphate, 0·1 N.

Starch solution—Prepare a 1 per cent. solution of soluble starch.

Procedure—

Accurately measure 10 ml of potassium dichromate solution into a specimen jar, place a glass triangle in the jar, and stand a Petri dish on the triangle. Accurately measure 2 ml of the body fluid into the Petri dish, cover the jar with a ground-glass plate, screw on the cap, and place in an incubator. After not less than 8 hours, remove the jar, allow it to cool, and remove the cap, plate and Petri dish. Rinse the legs of the triangle into the contents of the jar, and dilute these further to about 12 ounces with distilled water. Add 2 g of solid potassium iodide, and stir well. Titrate with 0·1 *N* sodium thiosulphate until nearly colourless, add a few millilitres of starch solution, and

continue the titration until colourless. Carry out a blank determination, omitting the body fluid, and calculate the concentration of alcohol in the sample (in milligrams per 100 ml of body fluid) by multiplying the difference (in millilitres) between the blank and sample titres by 57·5.

Normally, we prepare the jars last thing at night, leave them in the incubator overnight and titrate first thing in the morning.

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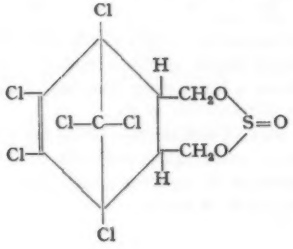
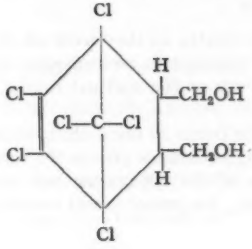
APPLICATION OF THE TOTAL ORGANIC CHLORINE METHOD TO THE DETERMINATION OF ENDRIN AND THIODAN* RESIDUES IN BLACKCURRANTS

The methods described by Sergeant and Thompson for the extraction, separation[1] and determination[2] of chlorinated hydrocarbon pesticide residues in plant material have been applied directly to endrin in blackcurrants. The recoveries of 0·98 p.p.m. of added endrin were 0·94 and 0·96 p.p.m., *i.e.*, 97 per cent., but at a lower level, 0·49 p.p.m. of added endrin, the recoveries were 0·39 and 0·32 p.p.m., *i.e.*, 72 per cent.

Thiodan, 6:7:8:9:10:10-hexachloro-1:5:5a:6:9:9a-hexahydro-6:9-methano-2:4:3-benzodioxathiepin-3-oxide, **I**, an insecticide developed in Germany,[3] has been examined in the United States[4,5] and elsewhere for use on edible crops. Technical thiodan contains two stereoisomers of similar insecticidal activity, thiodans A and B, present in the ratio of approximately 4 to 1. It also contains a variable, but small, amount of thiodan alcohol,[4] **II**, which is also the initial metabolic break-down product of thiodan; this alcohol contains no sulphur and is relatively non-toxic.



(I)



(II)

Chemical methods for determining thiodan residues, based on the determination of sulphur[5,6] or chlorine,[5] have been described; the former methods have the advantage of measuring only the toxic residue ascribable to unchanged thiodan, since any thiodan alcohol present will not be included in the result. This Note describes the application of Sergeant's method, modified in that the separation of thiodan in the extracted residue from thiodan alcohol and from interfering substances of plant origin is achieved by elution from activated alumina with benzene in place of hexane. In operation, the column is run "blind"; the preparation of the alumina is therefore standardised, and the solvents used must be dry.

Method

Procedure—

Extract 100 g of strigged blackcurrants with a mixture of 100 ml each of acetone and hexane (boiling range 68° to 69° C) or light petroleum (boiling range 60° to 80° C). Wash the extract, and concentrate it to about 1 ml of hexane as previously described.[1] Prepare activated alumina by heating aluminium hydroxide at 700° C for 8 hours, cooling and adding 5 per cent. of water. Prepare a column from 20 g of activated alumina slurried with benzene, cover with a 1-cm layer of granular anhydrous sodium sulphate, and wash with 20 ml of benzene. (The benzene should be dried by heating under reflux with sodium and then by distillation on to sodium.) Place the concentrated hexane extract on the prepared column, wash it in with 10 ml of benzene, and reject the first 10 ml of eluate. Continue elution at the rate of 2·0 to 2·5 ml per minute, adding a further 20 ml of

* The common name chlorthiepin has recently been approved for inclusion in British Standard 1831: 1957 ("Recommended Common Names for Pesticides").

TABLE I

RECOVERY OF THIODAN ADDED TO BLACKCURRANTS

Technical thiodan added, p.p.m.	Thiodan actually present, p.p.m.	Thiodan recovered, p.p.m.	Average recovery, %
0·50	0·46	0·43, 0·42	92
0·99	0·92	0·83, 0·89, 0·78	91
2·00	1·85	1·84, 1·83	99

benzene to the column and collecting the next 20-ml fraction. Add 2 drops of liquid paraffin, and remove the benzene from the fraction by evaporation in a stream of dry air at 40° C. Determine the total organic chlorine as described previously.[1,2]

A further 40 ml of benzene passed through the column failed to elute thiodan alcohol when 180 μg of this compound (prepared from thiodan) were added as such.

RESULTS

Technical thiodan assayed at 95·7 per cent., as $C_6H_8Cl_6O_3S$. When a column of alumina was used as described above to remove the thiodan alcohol, the thiodan content was found to be 92·5 per cent., so that the technical material contained some 3 per cent. of thiodan alcohol. The recovery of technical thiodan added to blackcurrants is shown in Table I.

This Note is published by permission of the Department of Scientific and Industrial Research.

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A FIELD TEST FOR NITROBENZENE VAPOUR IN AIR

PREVIOUSLY described methods for the detection and determination of nitrobenzene vapour in air have depended on the further nitration of the compound and then colorimetric determination of the resulting dinitrobenzene,[1] on reduction to aniline and then diazotisation and coupling[2,3] or on polarographic determination of the nitrobenzene, which is trapped in ethylene glycol.[4] However, none of these methods is a field test, since all require heat and other laboratory resources.

It was considered that a satisfactory method could be based on reduction to aniline, as in the method recommended by the International Union of Pure and Applied Chemistry,[3] provided that reduction could be accomplished in the cold. Gordon[5] described a qualitative test for nitrobenzene in aviation lubricants; it involved shaking the sample with liquid zinc amalgam and acid and coupling the resulting aniline, after diazotisation, with 2-naphthol. This method of reduction has been found to give satisfactory quantitative results when applied to solutions obtained by trapping nitrobenzene vapour in Cellosolve. No alternative method of determining aniline was entirely satisfactory under the conditions of this test. Hydrolysis of the diazotised aniline to yield phenol and then addition of Gibbs's reagent (2:6-dibromoquinone-chloroimide) to produce a blue colour is about as sensitive as coupling with disodium 2-naphthol-3:6-disulphonate (R salt), but heat is needed and the procedure is no less complicated. Condensation with furfural or *p*-dimethylaminobenzaldehyde, although simpler, is considerably less sensitive. Acidified solutions of nitrobenzene in Cellosolve can be reduced electrolytically at the zinc cathode of a simple cell by applying an e.m.f. of 1·5 volts supplied by a dry battery, but this mode of reduction was not adopted, as it is less well suited to a field test.

Method

Apparatus—

Bubbler—This should be of all-glass construction. The solvent container should be 9 cm deep and 1·3 cm in diameter, with a centrally blown bulb of diameter 2·5 cm. The internal diameter of the delivery tube should be 2 mm.

Pump—A metal hand-exhaust pump of capacity 120 ml or a mechanical pump capable of giving the correct rate of sampling (1500 ml per minute).

Trap—A suitable trap consists of a wide-necked bottle of capacity about 100 ml fitted with a 2-hole bung and delivery tubes and connected between the bubbler and the pump.

Tubes—Four 3-inch × 1-inch specimen tubes.

Pipette—A 5-ml dropping pipette with a rubber teat.

For use in the field, the apparatus may be mounted in a wooden case approximately 15 inches × 10 inches × 4 inches. Such a case also provides storage space for the reagent bottles and weighs about 7 lb.

Reagents—

All reagents should be of recognised analytical grade.

Cellosolve—2-Ethoxyethanol.

Hydrochloric acid—Dilute 5 ml of concentrated hydrochloric acid to 100 ml with water.

Sodium nitrite solution—Dissolve 3·5 g of sodium nitrite in 100 ml of water. The solution should not be kept for longer than 1 month.

Sodium carbonate solution—Dissolve 10 g of anhydrous sodium carbonate in 100 ml of water.

R salt solution—Dissolve 0·8 g of the purified sodium salt of 2-naphthol-3 : 6-disulphonic acid in 100 ml of boiling water, adjust the pH to about 8 (7·5 to 8·5) by adding *M* sodium carbonate, cool to room temperature, and filter. This reagent should be stored in darkness, and the solution should not be kept for longer than 1 month.

Ammonia solution—Dilute 20 ml of ammonia solution, sp.gr. 0·880, to 100 ml with water.

The reagents listed above can conveniently be stored in and dispensed from bottles having stoppers fitted with suitably graduated dropping pipettes.

Liquid zinc amalgam—Add 6 g of powdered zinc to 300 g of mercury and 5 ml of 20 per cent. v/v sulphuric acid. Stir, set aside for 2 hours, transfer to a separating funnel, and wash three times with dilute sulphuric acid. This reagent should be kept under a layer of dilute sulphuric acid and can conveniently be stored in and dispensed from a small polythene wash-bottle.

Colour standards—Dissolve 1·000 g of potassium dichromate in 1 litre of water; this is solution A. Dissolve 70·26 g of cobalt sulphate heptahydrate in 1 litre of water; this is solution B. Prepare the standards by placing in 3-inch × 1-inch specimen tubes the amounts of solutions A and B and water shown in Table I.

Table I

Composition of colour standards

Nitrobenzene concentration to be determined, p.p.m. v/v	Solution A in standard, ml	Solution B in standard, ml	Water in standard, ml
0·0	0·5	—	9·5
0·5	1·0	0·7	8·3
1·0	1·1	1·1	7·8
2·0	1·4	2·0	6·6

Procedure—

In the clean dry bubbler place 2 ml of Cellosolve, and connect the bubbler to the pump via the trap. Take a 6-litre sample of the air being tested at the rate of 1·5 litres per minute. (If the hand pump is used, 50 strokes at a rate of 5 seconds per exhaust stroke are needed.) Detach the bubbler, and add to its contents 1 ml of liquid zinc amalgam and then 4 ml of hydrochloric acid. Close the bubbler with a stopper, and shake vigorously for 1 minute. By means of the 5-ml teat-pipette, transfer 5·0 ml of the aqueous layer to a clean dry specimen tube, taking care to avoid any transference of zinc amalgam. Add 0·5 ml of sodium nitrite solution, shake gently to mix, and set aside

for 2 minutes. Add 2 ml of sodium carbonate solution, and then immediately add 0·5 ml of R salt solution. Shake to mix, add 2 ml of ammonia solution, shake again, and compare the colour of the solution with those of the colour standards. Alternatively, compare the colour of the solution with those of standard glass discs prepared for this test by Tintometer Ltd., Salisbury, Wilts.

Discussion of the method

Scope and precision—

The proposed method can be used for determining concentrations of nitrobenzene outside the limits 0·5 to 2·0 p.p.m. v/v by increasing or decreasing the size of the sample. The precision of the determinations is about ±20 per cent. A complete determination, including sampling, takes 10 to 15 minutes.

Interference—

The vapours of certain other aromatic nitro compounds and primary amines, if present, would presumably interfere; if aniline were present it could be determined before and after reduction, the difference being nitrobenzene.

Colour standards—

The colour standards were prepared by matching various mixtures of the inorganic salt solutions against the solutions resulting from determinations carried out on standard atmospheres of nitrobenzene; the atmospheres were produced by feeding an ethanolic solution of nitrobenzene of known concentration from a ram-operated syringe into a metered stream of air.[6] The concentrations of nitrobenzene in the atmospheres were verified from time to time by trapping in ethylene glycol and carrying out polarographic determinations. The inorganic colour standards are sufficiently stable for many weeks.

Bubbler—

The efficiency of the bubbler, as established by comparison between results for standard atmospheres and for standard solutions of nitrobenzene in Cellosolve, is about 85 per cent. at a sampling rate of 1500 ml per minute. Minor modifications, *e.g.*, the use of a capillary delivery tube, have not produced any marked change in efficiency. The use of two bubblers in series is not desirable in a field test; the method of calibration allows for an efficiency of 85 per cent.

Reduction by liquid zinc amalgam—

Absorptiometric comparisons between the colours produced by standard solutions of aniline that had been subjected to the entire procedure and those formed by equimolar standard solutions of nitrobenzene have shown that the reduction of amounts of nitrobenzene up to at least 12 μg by shaking with liquid zinc amalgam under the conditions described is substantially complete.

This Note is based on work carried out on behalf of the Committee on Tests for Toxic Substances in Air and the Ministry of Labour and is published by permission of the Department of Scientific and Industrial Research. I thank Mr. C. O. Harvey and Dr. D. I. Coomber for advice and criticism.

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FLAME-PHOTOMETRIC DETERMINATION OF SODIUM IN ALKALINE-EARTH CARBONATES

A MIXTURE of alkaline-earth carbonates is used in the valve industry to produce an oxide coating on the cathodes of certain types of valves. A typical method of manufacture is by precipitation from the mixed nitrate solution with sodium carbonate solution, and a method was required for determining the co-precipitated sodium impurity in manufactured carbonate mixture and also in the oxide coating of used cathodes. Schuhknecht and Schinkel[1] have described a flame-photometric method involving use of an instrument of fairly high wavelength selectivity and utilising the well known depressive effect of aluminium on calcium emission.

The object of our investigation was to develop a method for use with a simple filter-type instrument, *e.g.*, an E.E.L. flame photometer (Evans Electroselenium Ltd.), as described by Collins and Polkinhorne.[2]

EXPERIMENTAL

The mixture of alkaline-earth carbonates ("triple carbonate") used in this work contained 55 per cent. of barium carbonate, 41 per cent. of strontium carbonate and 4 per cent. of calcium carbonate.

Initial experiments with sodium-free solutions of calcium, barium and strontium confirmed that all three elements caused serious interference with emission from sodium. Attempts to suppress this interference by adding aluminium showed little promise, and a method in which the alkaline-earth metals were removed by precipitation with ammonium carbonate solution was investigated.

DEGREE OF CO-PRECIPITATION—

The theoretical disadvantage of the proposed method was the possibility of low recoveries, owing to co-precipitation of sodium. To check this possibility, a small amount of sodium-free "triple carbonate" was prepared from manufactured material by re-precipitation three times with ammonium carbonate solution. Equal weighed amounts of these dried carbonates were placed in small beakers, and known amounts of sodium were added. The carbonates were dissolved in a minimum volume of dilute nitric acid, the solutions were diluted to about 5 ml, and the alkaline-earth metals were re-precipitated with a known volume of ammonium carbonate solution. The insoluble carbonates were removed by centrifugation, the supernatant solutions and washings (after dilution to a definite volume) were sprayed into an E.E.L. flame photometer, and the scale readings were noted. The instrument was previously adjusted to full-scale deflection with use of the sodium filter and a solution containing 10 p.p.m. of sodium and the same amounts of nitric acid and ammonium carbonate as were present in the test solutions. The zero deflection was set by using the same amounts of acid and ammonium carbonate, with no added sodium.

Three series of solutions were examined. One series contained 0·25 g of sodium-free "triple carbonate," to which were separately added 0, 50, 100, 150, 200 and 250 μg of sodium, as a 0·01 per cent. solution of sodium chloride; the second series contained 0·025 g of "triple carbonate" and the same added amounts of sodium, and the third series contained only the added amounts of sodium, *i.e.*, no "triple carbonate" was present. Table I shows the galvanometer deflections obtained from these series of solutions.

These results indicated that no significant co-precipitation of sodium occurred when up to 0·25 g of "triple carbonate" was precipitated under the conditions stated. Further, they showed that the separation of alkaline-earth metals was effectively complete, as there was no increase in the deflection for sodium.

SENSITIVITY—

For a 0·25-g sample, a 5 per cent. deflection of the galvanometer corresponds to a sodium content of approximately 0·005 per cent. This was considered to be sufficiently sensitive for our purpose, although the useful limit could probably be decreased by using larger samples and smaller final volumes.

METHOD

PROCEDURE—

Dissolve a suitable weight of alkaline-earth carbonates or oxides in the minimum volume of 5 *N* nitric acid, and dilute the solution to about 5 ml with de-mineralised water. (The weight of

sample taken should not exceed 0·25 g and should not contain more than 200 μg of sodium.) Warm the solution to about 60° C, add 2·5 ml of a 10 per cent. aqueous solution of analytical-reagent grade ammonium carbonate, cool to room temperature, and set aside for 5 minutes. Separate the precipitate by centrifugation, wash it with cold water, and combine the supernatant solution and washings in a 100-ml beaker. Boil this solution to expel carbon dioxide, cool, dilute to 25 ml, and spray into the flame of an E.E.L. flame photometer previously set to full-scale deflection by using a standard solution containing 10 p.p.m. of sodium and the same concentrations of nitric acid and ammonium carbonate as are present in the test solution. Set the instrument to zero by using a solution containing the same amounts of reagents, but no added sodium.

Determine the sodium content of the sample by reference to a calibration graph plotted from the results obtained when solutions containing 0, 2, 4, 6, 8 and 10 p.p.m. of sodium and the same amounts of reagents used in the sample treatment are sprayed into the instrument. (This procedure compensates for the sodium content of the reagents, which normally corresponds to a scale reading of about 3 divisions.)

Results

The sodium content of a sample of "triple carbonate" was determined four times by the proposed procedure, and three further determinations were made after different amounts of sodium had been added. A 25-mg portion of the sample was used in each determination; the results were—

Sodium added, μg	Nil				20	40	60
Sodium found, μg	62·0	62·5	62·5	62·0	82·0	102·0	121·0

Table I

Galvanometer deflections for solutions containing various amounts of sodium

The volume of each solution used was 25 ml

Sodium content of solution, p.p.m.	Deflection in presence of— 0·25 g of "triple carbonate"	0·025 g of "triple carbonate"	Deflection in absence of "triple carbonate"
2	26	25	25
4	48	48	48
6	68	67	68
8	83	84	85
10	100	100	100

We thank the Directors of Associated Electrical Industries (Woolwich) Ltd. for permission to publish this Note.

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COLORIMETRIC DETERMINATION OF TRIPHENYLTIN RESIDUES

Triphenyltin acetate has been introduced commercially as a fungicide, *e.g.*, in controlling celery-leaf spot (*Septoria* spp.). The pure compound is a white micro-crystalline solid and melts at 124·5° C; it is insoluble in water, but soluble in the common organic solvents. Oral administration to rats showed an LD_{50} of 125 mg per kg of bodyweight; a daily administration of 50 p.p.m. retarded growth, and a strongly cumulative effect was observed.

The expected increase in the use of this fungicide, together with its rather high toxicity and its persistence, necessitated the development of an accurate method for determining residues on crops. Polarographic[1] and colorimetric[2] methods have been described and involve extraction, purification and destruction of the triphenyltin acetate. An alternative method is reported here and is based on direct determination of triphenyltin acetate, so avoiding the rather laborious destruction of the compound. Aldridge and Cremer's method,[3] in which dithizone was used as a

reagent for triethyltin compounds, has been applied. The properties of the triphenyltin - dithizone complex closely resemble those of the triethyltin - dithizone complex, and the absorption spectra of the two complexes are similar. It can be seen from Fig. 1 that maximum absorption for the triphenyltin - dithizone complex is at 450 mμ and that the complex has the same absorption as dithizone at 510 mμ.

Experimental

The sample of plant material is extracted with methylene chloride to remove triphenyltin acetate, the extract is evaporated to dryness, and the residue from the evaporation is dissolved in chloroform. Interfering cations, *e.g.*, cupric, ferric, lead and stannic, are removed by shaking the chloroform solution with a buffer solution (pH 8·4) containing ethylenediaminetetra-acetic acid. A solution of dithizone in chloroform is then used to determine the tin compound quantitatively.

The determination of triphenyltin compounds in plant extracts necessitates thorough purification of the extracts, as other extracted materials inhibit formation of the complex. Treatment with Brockmann's alumina (after being heated at 1000° C for 1½ hours) *plus* 3 per cent. of added water is used for this purpose; the alumina completely adsorbs the inhibiting substances, leaving the triphenyltin acetate in solution. (Diphenyltin compounds, which may be present in the solution and interfere with the colorimetric determination, are also adsorbed.) If much chlorophyll is present in the extract, it is not completely removed by the column of alumina, but the addition to the column of a small layer of infusorial earth ensures complete adsorption.

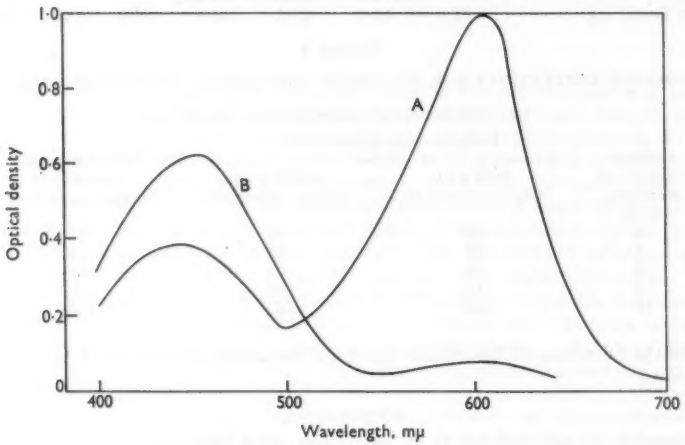

Fig. 1. Absorption spectra of dithizone and the triphenyltin - dithizone complex: curve A, 67 μg of dithizone in 10 ml of chloroform; curve B, 67 μg of dithizone and excess of triphenyltin acetate in 10 ml of chloroform. Optical densities measured in 1-cm cuvettes

Method

Reagents—

Methylene chloride—Distil before use.

Chloroform—Shake 1 litre of laboratory-reagent grade chloroform with 75 ml of an aqueous 5 per cent. solution of sodium phenate, wash with water, and distil. Discard the first and last 50-ml portions of distillate.

Sodium sulphate, anhydrous.

Alumina—Heat Brockmann's alumina at 1000° C for 1½ hours, allow to cool in a desiccator, and add 3 per cent. w/w of water before use.

Infusorial earth.

Buffer solution, pH 8·4—Dissolve 19 g of sodium tetraborate decahydrate, 12 g of boric acid and 2·5 g of disodium ethylenediaminetetra-acetate in water, and dilute to 1 litre.

Dithizone solution—Prepare a 0·004 per cent. w/v solution of purified[4] dithizone in chloroform.

PROCEDURE—

Extract 150 g of celery with 300 ml of methylene chloride. Place 10 to 50 ml of extract in a 100-ml beaker, and remove the solvent by evaporation at room temperature. Dissolve the residue in 6 ml of chloroform, and dry the solution over anhydrous sodium sulphate.

Partly fill a chromatographic tube (internal diameter 5 mm) with chloroform, add 0·5 g of alumina, and pack the column by tapping. Pour the chloroform solution of the residue on to the freshly prepared column of alumina immediately after the chloroform layer above the alumina has disappeared. Discard the first 1-ml portion of eluate, and collect the next 3 ml in a graduated 10-ml cylinder fitted with a tap and a glass stopper.

To the contents of the cylinder add 5 ml of buffer solution, shake for 1 minute, add 2 ml of dithizone solution, and shake for a further 1 minute. Allow the layers to separate, and transfer the chloroform layer to a 1-cm cuvette.

Measure the optical density of the chloroform layer from a similarly treated reagent blank at 610 mμ, and use the chloroform layer from the fungicide-containing sample as reference solution.

Note that chlorophyll, present in the eluate from the column, may interfere with the determination. It can be easily removed by placing a small amount of infusorial earth on the top of the alumina; it is advisable to apply pressure to the top of such a column to obtain a convenient flow of solution.

PREPARATION OF CALIBRATION GRAPH—

Prepare a standard chloroform solution containing 20 μg of triphenyltin acetate per ml, and use this standard to prepare solutions containing from 0 to 60 μg of triphenyltin acetate in 3 ml of chloroform. To each of these solutions add 5 ml of buffer solution and 2 ml of dithizone solution, and measure the optical densities as described above. The calibration graph should be linear and pass through the origin.

RESULTS

Known amounts of triphenyltin acetate added to extracts of celery that had not been treated with this fungicide were recovered by the proposed method, all optical measurements being made with a Unicam SP500 spectrophotometer; the results were—

Triphenyltin acetate added, μg	12·5	25	25	25	25	50	50	75	
Triphenyltin acetate found, μg ..	11·9	22·6	25·3	24·0	24·0	44·0	51·0	76·1	
Recovery, % ..	95	90	101	96	96	88	102	101	(Mean 96

The method is sensitive to about 0·1 p.p.m. of triphenyltin acetate.

We thank Professor G. J. M. van der Kerk, Utrecht, for kindly providing us with pure samples of triphenyltin acetate and Drs. E. J. Miller and N. A. Smart, of the Plant Pathology Laboratory, Harpenden, for their valuable advice in preparing the manuscript for publication.

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DETERMINATION OF LOW CONCENTRATIONS OF ALUMINIUM AND CHROMIUM

A METHOD for determining trace amounts of aluminium and other impurities has been described[1]; the aluminium was precipitated, together with a known amount of lanthanum as carrier, by ammonium hydroxide and hydrogen peroxide. The hydroxide precipitate was then dried, ignited at 1000° C and examined by d.c. arc emission spectrography. The concentration of aluminium was determined by measuring the logarithm of the intensity ratio of the aluminium line at 2568·0 A to the lanthanum line at 2855·9 A and reading the concentration from a working curve prepared

from a series of solutions containing known ratios of aluminium to lanthanum. The method has been in routine use in this laboratory for several years for examining samples of magnesium, calcium and thorium. During this period, five additional facts have been ascertained.

(*i*) Nitrate causes precipitation of lanthanum to be incomplete; because of this, lanthanum chloride is the best commercial salt to use. If oxidation with nitric acid is essential for the removal of organic reagents, nitrate must be removed by subsequent heating to fumes.

(*ii*) Quantitative precipitation of lanthanum is assisted by beginning precipitation from a strongly acid solution and then adding a saturated solution of ammonia; generally, 30 ml of concentrated hydrochloric acid are added just before precipitation. If a large weight of magnesium (10 g) is being examined, 10 g of ammonium chloride are also added, and more ammonium chloride is used for even larger samples.

(*iii*) To avoid the necessity of continuously drawing working graphs, a permanent one can be plotted from the results obtained for triplicate samples. The graph is corrected for residual aluminium in the lanthanum chloride by any of the standard methods,[2,3] and it is then only necessary to determine the reagent blank value and to check the working graph at infrequent intervals.

(*iv*) When 50 mg of lanthanum are used, the effective lower limit for a 10-g sample is 0·1 p.p.m. of aluminium.

(*v*) Chromium can be simultaneously determined to a lower limit of 0·05 p.p.m. by measuring the logarithm of the intensity ratio of the chromium line at 2835·6 A to the lanthanum line at 2855·9 A. This was shown by using prepared solutions of known composition and samples previously analysed colorimetrically by the diphenylcarbazide method.

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THE DETERMINATION OF BORON IN ALUMINIUM AND SILICON - ALUMINIUM ALLOYS

Aluminium and silicon - aluminium alloys containing 0·5 to 1 per cent. of boron are finding increasing use in the semi-conductor industry. Determination of the boron content by conventional methods involving distillation of methyl borate is complicated by the fact that the boron is present in a variety of forms, not all of which are soluble in mineral acid. Fusion with sodium peroxide of either the original sample of alloy or the insoluble residue from an acid attack results in dilute solutions for distillation. Further, all glassware must be boron-free and this is not readily available.

A direct colorimetric procedure in which these difficulties are avoided has been devised; it is based on a method proposed for determining boron in steel.[1] In this procedure the use of glassware has been largely avoided, the exception being the use of calibrated flasks for a short time with acid solutions; pick-up of boron under these conditions is extremely slight.

Method

Reagents—

All reagents should be of recognised analytical grade.

Sulphuric acid, sp.gr. 1·84.
Sulphuric acid, diluted (4 + 1).
Sulphuric acid, 2 N.
Sodium carbonate solution, 10 *per cent. w/v*—Store in a polythene bottle.
Sodium peroxide.
Cation-exchange resin—Zeo-Karb 225 (−60 to +100 mesh) in the hydrogen form.
Dianthrimide reagent solution—Dissolve 0·4 g of 1 : 1′-dianthrimide in 100 ml of sulphuric acid, sp.gr. 1·84. Store this stock solution in a lightly stoppered bottle in a desiccator. For use, transfer 5 ml of the solution to a dry 100-ml calibrated flask, and dilute to the mark with concentrated sulphuric acid.

Standard boron solution—Dissolve 0·228 g of boric acid in 1 litre of water, and dilute 10 ml of this solution to 100 ml with water.

1 ml ≡ 4 μg of boron.

PROCEDURE—

From the sample in the form of filings or fine sawings, weigh 0·1 g into a nickel crucible, and mix with 2 g of sodium peroxide. Allow the mixture to sinter for 30 minutes on an asbestos mat over a bunsen burner, and then fuse over the free flame. After solidification, leach the melt with water in a polythene beaker, cool, transfer to a 250-ml acrylic-resin measuring cylinder (obtainable from A. Gallenkamp Ltd.), make up to 250 ml, and return to the beaker. Allow the precipitated hydrated nickel oxide to settle, and then transfer a 10-ml aliquot of the solution, by means of a Perspex pipette (obtainable from X-Lon Products Ltd.), to a small polythene beaker or platinum vessel. Treat the solution with 2 *N* sulphuric acid, dropwise, until its pH is approximately 2, as judged by the use of Johnson's universal-indicator paper, and then transfer to a 7-inch × 1-inch column of cation-exchange resin contained in a polythene tube. Elute with 250 ml of water, and collect the effluent in a polythene beaker. Make the effluent faintly alkaline by adding 15 ml of 10 per cent. sodium carbonate solution, check that the pH is between 8 and 9, and evaporate to dryness in a platinum dish.

Allow the residue to cool, dissolve it in 7·5 ml of water, and add 7·5 ml of diluted sulphuric acid (4 + 1) rapidly to avoid overheating. Transfer the solution to a 20-ml calibrated flask, and use diluted sulphuric acid (4 + 1) for rinsing the dish and diluting the contents of the flask to volume. At this stage, the flask should contain the assay solution in diluted sulphuric acid (1 + 1). Transfer a 5-ml aliquot to a 50-ml calibrated flask, add 5 ml of dianthrimide reagent solution, dilute to the mark with sulphuric acid, sp.gr. 1·84, transfer to a silica boiling tube, and cover with a loosely fitting silica cover. Heat the solution at 95° ± 1° C for 16 hours, cool to room temperature, and measure the optical density in a 4-cm cell at 640 mμ with a Unicam SP500 spectrophotometer or similar instrument. Determine boron by reference to a standard graph prepared as described below.

When the alloy is known to be free from interfering elements, such as chromium and vanadium, the ion-exchange procedure can be omitted. With such samples, take a 2·5-ml aliquot from the original 250 ml of solution, add 10 ml of sulphuric acid, sp.gr. 1·84, and then 5 ml of dianthrimide reagent solution, dilute to 50 ml with sulphuric acid, sp.gr. 1·84, and develop the colour as described above.

PREPARATION OF STANDARD GRAPH—

Place 0,1-, 1·5- and 2-ml portions of standard boron solution in separate polythene beakers, dilute each to 2·5 ml, and add 10 ml of sulphuric acid, sp.gr. 1·84. Cool, transfer the solutions to 50-ml calibrated flasks, add 5 ml of dianthrimide reagent solution to each, and dilute to the mark with sulphuric acid, sp.gr. 1·84. Treat these solutions as described above, measure the optical densities at 640 mμ, and plot a graph of optical density against boron content.

REFERENCE

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JOHNSON, MATTHEY & CO. LIMITED
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EXHIBITION GROUNDS
WEMBLEY, MIDDLESEX

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Received *May 2nd*, 1960

A NOTE ON VANADIUM POISONING

THERE appear to be few recorded cases of vanadium poisoning in man and this Note may be of interest on this account.

Two workmen were chipping an incrustation from a tank, and after 4 to 6 hours' inhalation of the dust they reported to the factory first-aid station with symptoms of nausea and vomiting. The tongues of both men were blue-black in colour and blue lines were seen around their gums. Other symptoms described included sore throat, cough, dyspnoea and in one case depression. The men rapidly improved when they were taken off this work. The symptoms resembled those described by Williams[1] and Sjoberg.[2] Samples of urine from the two men contained 2 p.p.m. of vanadium, but full 24-hour samples of urine and faeces were not available.

The incrustation contained calcium, aluminium, phosphorus, fluorine, vanadium and silicon and was strongly alkaline in reaction, as the tank was used for treating bauxite with sodium hydroxide. Lead and other poisonous metals were present in significant amounts, but the vanadium content of the material was found to be 3·5 per cent.

Vanadium was determined in the incrustation by fusion and acidification, as recommended by Meyer and Pawletta,[3] and in the urine by evaporation, ashing, solution in 20 per cent. sulphuric acid and then matching the red colour produced with hydrogen peroxide, according to the same method. The presence of vanadium was confirmed by the blue-black colour produced with tannin.[4]

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Book Reviews

Chromatographic Reviews: Progress in Chromatography, Electrophoresis and Related Methods. Volume II. Edited by Michael Lederer. Pp. viii + 195. Amsterdam, London, New York and Princeton: Elsevier Publishing Co.; London: D. Van Nostrand Co. Ltd., 1960. Price 46s.

The first volume of Chromatographic Reviews was accorded a somewhat grudging welcome to our overburdened library shelves. The quality of the subject matter and the production were highly praised, but one doubted if the editor could repeat such a performance annually. The second volume puts our fears to rest, at least for a time; true it is 80 pages shorter than volume I and has 6 instead of 9 reviews, but these are at least as well written and useful as before. All are taken from the *Journal of Chromatography*, as previously, but have been translated when necessary into adequate and usually excellent English. Future volumes may include reviews not previously published in the *Journal*.

Volume II opens with a condensed but comprehensive review of gas chromatography, by C. J. Hardy and F. H. Pollard, citing over 600 references. In retrospect, it is astonishing that a technique so valuable to industry as is gas - liquid chromatography can have lain dormant for 10 years since it was first foreshadowed by Martin and Synge in 1941. Its versatility is illustrated by the statement that quantitative separations can be effected within a few hours (sometimes minutes) of complex mixtures in amounts from 10^{-15} to 70 g and boiling over the range $-200°$ to 400° C. H. Bloemendal provides a brief but adequate and well illustrated account of starch-block electrophoresis, covering design, performance of equipment and applications, mainly to proteins and peptides, including hormones and enzymes. G. Biserte *et al.* provide the longest chapter, on paper chromatography of dinitrophenylamino acids. This differs in providing full experimental details and mentioning all the numerous snags in the application of this technique to the analysis of proteins or determination of terminal amino acids. J. B. Harborne, who reviewed the chromatography of anthocyanins in volume I, here completes the picture with an excellent chapter on the flavonoid pigments. Paper chromatography with markers, in one or two dimensions, and visualisation in ultra-violet light and with sprays will often suffice to identify these pigments in plant extracts. Numerous tables of R_g values are included. Moreover, chromatography of a new compound and its hydrolysis products in several solvent systems gives valuable clues to its structure and consumes only 1 mg or so. Preparative methods are also briefly reviewed.

H. K. Prins writes a fascinating account of the separation of human haemoglobin types. He notes meticulously the pros and cons of moving-boundary and zone electrophoresis on paper, cellulose acetate and starch blocks and columns, chromatography on IRC-50 resin or carboxymethyl cellulose, besides alkali-denaturation and solubility methods. Since the 13 or more types probably differ only by a few amino acid residues, it is not surprising that the results do not tally exactly

and that it is recommended that at least two techniques should be used in diagnostic applications. The final chapter, by E. Hayek, deals with adsorption and precipitation chromatography on alumina and other materials. It deals with mechanisms rather than applications and is rather disappointingly inconclusive.

This volume must be recommended as an indispensable work of reference for those engaged in any of these fields.

E. LESTER SMITH

CHEMICAL ANALYSIS OF CAST IRON AND FOUNDRY MATERIALS. By W. WESTWOOD, B.Sc., A.I.M., and A. MAYER, B.Sc., F.R.I.C. Second Edition. Revised by W. E. CLARKE, F.R.I.C. and H. GREEN. London: George Allen and Unwin Ltd. 1960. Price 63s.

Since the first edition of this book was published, in 1951, it has become widely recognised as a standard reference work on the analysis of cast iron and associated foundry materials. In this edition, the general lay-out and treatment of the subject are on similar lines to those of the first edition, but the subject matter has been revised and brought completely up to date.

Many of the original methods remain unchanged, understandably so, as most of them are authoritative classical procedures for which the passage of time has done nothing to impair their status or validity. Some methods have been modified in matters of detail, for example, those for sulphur, for titanium in alloy cast iron and for cerium in nodular graphitic iron. New methods are described for aluminium, antimony, lead, phosphorus and vanadium. In introducing these and in modifying some of the older methods, full account has been taken of recent developments in the use of solvent-extraction procedures and selective colour reactions and in the application of spectrophotometric and other instrumental techniques.

The choice of methods is excellent and no one will go far wrong in following the authors' recommendations. It is perhaps a pity that the periodate method for manganese was not given in preference to the persulphate method. The periodate method is now generally recognised as being the best of all those available for the determination of this element, and the authors themselves state in the general discussion of methods that periodate produces a more stable permanganate, but content themselves with giving a single very old reference to the method and make no mention of the British Standard produced in 1951. There are also one or two other well recognised British Standard procedures that might have been detailed or at least brought to notice.

The remaining sections of the book give a comprehensive treatment of the analysis of auxiliary raw materials and products associated with the iron-foundry industry, covering such materials as ferro-alloys, iron ore, cupola and blast-furnace slags, silica and alumino-silicate refractories, coke, coal dust and linseed oil. The section on slags includes a completely new scheme of rapid methods of analysis by which the complete analysis of a cupola slag can be made in less than one working day. It must be said, however, that section VI, on refractory materials, is too much on traditional lines and does not take account of specialist methods in this field that have been developed in recent years, mainly as a result of co-operative work sponsored by such bodies as the British Ceramic Research Association and the Society of Glass Technology.

The over-riding importance of this book is that in scope and usefulness it goes much beyond its title. It is by title a book for the iron-foundry chemist, but most of the methods described are equally suitable for the analysis of steel, with little or no modification. It is therefore a book for the iron and steel industry as a whole, and in this respect it demands the serious attention of everyone interested in ferrous analysis, for it fills an essential need in a field where the few other existing text-books are either inadequate or out of date.

B. BAGSHAWE

HETEROMETRY. By MORDECHAI BOBTELSKY. Pp. viii + 229. Amsterdam, London, New York and Princeton: Elsevier Publishing Co.; London: D. Van Nostrand Co. Ltd. 1960. Price 42s.

Heterometry is based on a simple conception. A substance in solution is titrated with a reagent with which it forms a precipitate, optical-density measurements are made during the titration, and a curve is drawn to correlate optical density with volume of titrant. In most analytical applications the end-point is indicated by a sharp break in the curve when the point of

maximum turbidity is reached. This principle is applied to the determination of many of the commoner metals, either with well known inorganic precipitants or, more usually, with complex-forming organic reagents. Many of these titrations are conducted with solutions of concentration ranging down to 10^{-5} *M*, and the accuracy achieved is often better than 99 per cent.

Many heterometric curves exhibit several "critical points" or inflexions, and these yield information on intermediate products formed during the titration. For example, in the titration of bismuth with amidopyrine in the presence of iodide, evidence of the formation of ten different compounds is obtained. Seventy-five pages are devoted to the study by this means of complexes formed by metals with dicarboxylic acids, hydroxycarboxylic acids, metaphosphates, polyphosphates, thio compounds and dyes.

Most of the work is from the many papers already published from Professor Bobtelsky's department at the Hebrew University of Jerusalem. The original publications seem to have attracted little attention, and it is to be hoped that the collective work will create the interest that the subject undoubtedly merits. The equipment required is simple and inexpensive, the methods are generally rapid, and, in the study of complexes, they yield information that would not so readily be obtained by other means.

The chemical presentation and the English are both at times a little unconventional, and the latter is occasionally rather obscure. The language difficulty cannot excuse the mis-naming of ferrocyanide as ferrous cyanide on p. 36. Otherwise, the compilation bears the mark of enthusiasm tempered with great care. The publishers have also made an excellent job of the rather intricate tables and graphs as well as of the text.

W. C. JOHNSON

ANALYTICAL CHEMISTRY OF TITANIUM METALS AND COMPOUNDS. By MAURICE CODELL. Pp. xiv + 378. New York and London: Interscience Publishers Inc. 1959. Price $12.00; 90s.

It is well within the lifetime of most of us that titanium was classified amongst the rare metals, but to-day this concept has changed. Titanium is now recognised to be one of the most universally distributed elements, a recognition that has stemmed from two main outlets; the use of titania for pigmentation purposes and the ever-increasing variety of commercial purposes for which titanium metal is admirably suitable.

Any analytical publication that collates existing knowledge on these two related fields and aims at bringing the analyst up to date must, therefore, be welcomed. This publication is the ninth of a series of monographs on analytical chemistry and its applications, and the analytical standard of the series is maintained. The reader has only to scan the list of editors and assistant editors and to know the international status of the author to be encouraged to read the detailed information contained in this book.

Sections are logically divided under four main headings: (*a*) General procedures, including basic fundamental reactions, (*b*) Determination of metallic elements in titanium and titanium alloys, (*c*) Determination of non-metallic elements in titanium and titanium alloys and (*d*) Analysis of titanium-base materials.

In the development of any analytical procedure for application to a new or existing commercial product, the analyst immediately thinks in terms of proven procedures, on this occasion ranging from the use of diphenylcarbazide for chromium, dimethylglyoxime for nickel and neocuproine for copper to the application of standard polarographic procedures for specified metals. In this respect, development of the recommended procedures for application to titanium and its associated metallurgical products has followed customary practice; nevertheless, it is disappointing to note that the determination of lead involves the formation and final weighing of lead sulphate.

The chapter devoted to "vacuum-line methods" is comprehensive, but emphasis on the relative merits of each method could have been profitably stressed. Some analysts are biassed in favour of macro vacuum fusion methods, with the obvious advantage of being able to use a relatively large weight of sample, but the speed associated with micro, or perhaps more correctly, semi-micro, vacuum fusion methods is unquestionable, and this aspect, in addition to supplying information relative to homogeneity of the material, merits more detailed comment. Vacuum fusion methods, with all their advantages, involve costly equipment, and the present state of knowledge in the field of carrier-gas techniques (in which much cheaper equipment is used), and possible trends in so-called chemical halogenation procedures are not adequately discussed. The determination of

nitrogen by vacuum-line methods and controversial views on the reliability of nitrogen values obtained by such methods are other aspects not brought out.

In any procedure of which the determination of a minor constituent is the ultimate objective, the quality of the reagents is of paramount importance, and this is normally implied, but in the recommended method for determining calcium (p. 225) the quality of ammonium hydroxide and nitric acid, surprisingly, merits special mention; the simple expedient of preliminary distillation is recommended, a straightforward treatment normally carried out by the supplier.

There are not many factual statements to which exception can be taken, but the comment that "oxygen is present in titanium metals as the lower oxide TiO, rather than TiO_2" is misleading because it is well established that large amounts of interstitially dissolved oxygen, up to 15 per cent. w/w, can be present in these materials.

The section devoted to titanium and titanium alloys is singularly free from any reference to work in allied fields in this country, where significant contributions have been made and much information published on the subject; this aspect of the book is most disappointing. Methods recommended for the analysis of titanium-base materials are, presumably, those in current use in the Pigments Department of E.I. du Pont de Nemours.

Those sections dealing with the analysis of titanium tetrachloride, titanium-bearing minerals and ore concentrates are comprehensive and should be readily adaptable to suit particular circumstances, although the statement on page 339, and elsewhere, that vanadium and chromium are usually present in very low concentrations should be accepted with considerable reserve.

It seems almost inevitable that any new publication will contain some unintentional errors; it is perhaps not surprising, therefore, that on separate occasions, on pages 308 and 310, lithopone is spelt incorrectly, and in the same section the composition of lithopone is twice referred to as $ZnS–PbSO_4$.

Although experts might agree that the book contains little that was not already known and maintain that the best and latest information on the subject had not been published, the book can be recommended for the laboratory just embarking on any aspect of analytical chemistry in which titanium plays a predominating part.

W. T. ELWELL

FOOD POISONING. By ELLIOT B. DEWBERRY, M.B.E. Fourth Edition. Pp. xvi + 411. London: Leonard Hill (Books) Ltd. 1959. Price 45s.

The fourth edition of "Dewberry" makes a welcome appearance for those who needs must be concerned with maintaining high standards for the nation's foods. Since the third edition appeared in 1950, it is coincident that there has been a steady upgrading in the care taken generally to protect the consumer from the risk of food poisoning, despite sporadic local outbreaks. This is undoubtedly partly due to the vigilance of the health inspectors collaborating with public analysts, and I cannot think other than that books of the nature of Dewberry have contributed to this trend.

This edition is virtually a new book, since it has been practically re-written as a result of the author having consulted a wide horizon of new sources of references. The recent findings of bacteriologists, epidemiologists, toxicologists and medical officers of health have been studied and the relevant material incorporated into the text. Nevertheless, as is pointed out in the book, of the outbreaks that do occur, made-up meals and meat dishes are the principal offenders—usually due to *Salmonella*, *Staphylococcus aureus* or *Clostridium welchii*.

Bacteriological contamination, sources and mode of infection, and prevention and control largely make up the material of the book. Nevertheless, other means and methods of food poisoning are also minutely described, *e.g.*, poisoning due to fungi and higher plants, poisonous fish and metallic salts. From the analyst's viewpoint, the lay-out of the book is interesting in that it devotes specific chapters to individual types of poisoning, *e.g.*, staphylococcal poisoning and botulism, thus making it easy to find relevant information on the subject. But possibly the most useful chapter is devoted to laboratory investigation of food-poisoning cases. The method of taking samples and the types of media to be used are given in considerable detail. Two useful appendixes deal with (*a*) steps to be taken by medical officers of health in the control of food poisoning and (*b*) methods for control of enteritis, particularly at sea.

The book is well indexed and has a good bibliography. The publishers might have used better paper and given the diagrams and photographs more definition. These defects spoil the presentation.

R. F. MILTON

Publications Received

ANNUAL REPORTS ON THE PROGRESS OF CHEMISTRY FOR 1959. Volume LVI. Pp. xiv + 476. London: The Chemical Society. 1960. Price 40s.

BRITISH PHARMACOPEIA 1958: ADDENDUM 1960. Pp. xxii + 83. London: The Pharmaceutical Press for The General Medical Council. 1960. Price 30s.

ANALYTICAL APPLICATIONS OF DIAMINO-ETHANE-TETRA-ACETIC ACID. By T. S. WEST and A. S. SYKES. Second Edition. Pp. 127. Poole, Dorset: The British Drug Houses Ltd. 1960. Price 4s. 6d.

TINPLATE TESTING: CHEMICAL AND PHYSICAL METHODS. By W. E. HOARE, D.Sc., F.I.M., and S. C. BRITTON, M.A., F.R.I.C., F.I.M. Pp. 55. Greenford, Middlesex: Tin Research Institute. 1960. Gratis.

THE USE OF ORGANIC REAGENTS IN INORGANIC ANALYSIS. By A. I. BUSEV and N. G. POLIANSKII. Translated from the Russian by J. T. GREAVES, M.A. Pp. viii + 76. Oxford, London, New York and Paris: Pergamon Press Ltd. 1960. Price 21s.

LABORATORY HANDBOOK OF TOXIC AGENTS. Editor-in-Chief: C. H. Gray, D.Sc., M.D., F.R.C.P., A.R.C.S., F.R.I.C. Pp. viii + 170. London: The Royal Institute of Chemistry. 1960. Price 20s.

THE RADIOCHEMISTRY OF INDIUM. By D. N. SUNDERMAN and C. W. TOWNLEY. Pp. vi + 46. Washington, D.C.: National Academy of Sciences—National Research Council. 1960. Price 50 cents.

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PURE AND APPLIED CHEMISTRY. (The Official Journal of the International Union of Pure and Applied Chemistry.) Volume I, No. 1, 1960. Scientific Editor: B. C. L. WEEDON. Pp. 186. London: Butterworths Publications Ltd. Subscription price per volume (published irregularly, four issues per volume) 120s.; $18.00.

A new journal.

PRINCIPLES OF PHYSICAL CHEMISTRY. By WILLIAM H. HAMILL and RUSSELL R. WILLIAMS, jun. Pp. xii + 607. Edinburgh and London: Oliver and Boyd Ltd. 1960. Price 30s.

REPORTS ON THE PROGRESS OF APPLIED CHEMISTRY. Volume XLIV: 1959. Editor: H. S. ROOKE, M.Sc., F.R.I.C. Pp. 705. London: The Society of Chemical Industry. 1960. Price 140s.

ADVANCES IN FLUORINE CHEMISTRY. Volume I. Editors: M. STACEY, F.R.S., J. C. TATLOW, Ph.D., D.Sc., and A. G. SHARPE, M.A., Ph.D. Pp. viii + 203. London: Butterworths Publications Ltd. 1960. Price 45s.

REPRINTS FROM *THE ANALYST*

REPRINTS of the following papers published in *The Analyst* are now available from the Assistant Secretary, The Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1 (not through Trade Agents). Orders MUST be accompanied by a remittance for the correct amount made out to The Society for Analytical Chemistry.

Analytical Methods Committee: Metallic Impurities in Organic Matter Sub-Committee.

"The Determination of Small Amounts of Arsenic in Organic Matter" (September, 1960). Price (members) 1s. 6d.; (non-members) 2s. 6d.

"Methods for the Destruction of Organic Matter" (September, 1960). Price (members) 1s. 6d.; (non-members) 2s. 6d.

Review Paper.

"The Application of Atomic Absorption to Chemical Analysis," by D. J. David (this issue; available shortly). Price 5s.

Erratum

OCTOBER (1960) ISSUE, p. 714, title of paper, 2nd line of synopsis and 1st line of text of paper.

For "17-Ketosteroids" *read* "17:21-Dihydroxy-20-oxosteroids."